LABORATORY GUIDELINES, EQUIPMENT, AND STOCK SOLUTIONS

Laboratory Safety

Persons carrying out the protocols in this manual may encounter the following hazardous or potentially hazardous materials (1) pathogenic and infectious biological agents and (2) toxic chemicals and carcinogenic, mutagenic, or teratogenic reagents (Table A.2A.1). Most governments regulate the use of these materials; it is essential that they be used in strict accordance with local and national regulations. Cautionary notes are included in many instances throughout the manual, and some specific guidelines are provided below (and in references therein). However, we emphasize that users must proceed with the prudence and precautions associated with good laboratory practice, under the supervision of personnel responsible for implementing laboratory safety programs at their institutions. Guidelines for the safe use of radioisotopes are presented in *APPENDIX 2B*.

SAFE USE OF BIOHAZARDS AND INFECTIOUS BIOLOGICAL AGENTS

Precautions described in this section should be applied to the routine handling of viable pathogenic microorganisms, as well as all human-derived materials, because they may harbor dangerous pathogens such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), and a host of bacterial pathogens. In addition to the guidelines provided herein, experimenters can find a wealth of information about handling infectious agents in the government publications *Biosafety in Microbiological and Biomedical Laboratories* and *Working Safely with HIV in the Research Laboratory* (see Literature Cited).

Routine Precautions When Working with Biohazards

The following practices are recommended for all laboratories handling potentially dangerous microorganisms, whether pathogenic or not:

1. Decontaminate all work surfaces after each working day using an appropriate disinfectant. Decontaminate all spills of viable material. See discussion under Disinfectants for Biohazards.
2. Decontaminate all liquid or solid wastes that have come in contact with viable material.
3. Do not pipet by mouth.
4. Do not allow eating, drinking, smoking, or application of cosmetics in the work area. Do not store food in refrigerators that contain laboratory supplies.
5. Wash hands with disinfectant soap or detergent after handling viable materials and before leaving the lab. Do not handle telephones, doorknobs, or other common utensils without disinfecting hands.
6. When handling viable materials, minimize creation of aerosols.
7. Wear lab coats (preferably disposable) when in work area, but do not wear them away from the work area.
8. Wear disposable latex gloves when handling viable materials. These should be disposed of as biohazardous waste. Change gloves if they are directly contaminated.

Contributed by George Lunn and Gretchen Lawler
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Table A.2A.1 Commonly Used Hazardous Chemicals[a]

Chemical	Hazards	Remarks[b]
Acetic acid, glacial	Corrosive, flammable liquid	
Acetonitrile	Flammable liquid, teratogenic, toxic	
Acridine orange	Carcinogenic, mutagenic	See Basic Protocol 2
Acrylamide	Carcinogenic, toxic	Use dust mask; polyacrylamide gels contain residual acrylamide monomer and should be handled with gloves; acrylamide may polymerize with violence on melting at 86°C
Alcian blue 8GX		See Basic Protocol 2
Alizarin red S (monohydrate)		
p-Amidinophenylmethanesulfonyl fluoride (APMSF)	Enzyme inhibitor	See Basic Protocol 11
7-Aminoactinomycin D (7-AAD)	Carcinogenic	
4-(2-Aminoethyl)benzenesulfonyl fluoride (AEBSF)	Mutagenic, enzyme inhibitor	See Basic Protocol 11
Ammonium hydroxide, concentrated	Corrosive, lachrymatory, toxic	
Azure A	Mutagenic	See Basic Protocol 2
Azure B	Mutagenic	See Basic Protocol 2
Benzidine (BDB)	Carcinogenic, toxic	See Basic Protocol 1
Bisacrylamide	Toxic	
Boron dipyrromethane derivatives (BODIPY dyes)	Toxic	
Brilliant blue R	Carcinogenic, mutagenic	See Basic Protocol 2
5-Bromodeoxyuridine (BrdU)	Mutagenic, teratogenic, photosensitizing	
Cetylpyridinium chloride (CPC)	Toxic	
Cetyltrimethylammonium bromide (CTAB)	Corrosive, teratogenic, toxic	
Chloroform	Carcinogenic, teratogenic, toxic	
Chlorotrimethylsilane	Carcinogenic, corrosive, flammable liquid, toxic	Reacts violently with water; see Basic Protocol 3
Chromic/sulfuric acid cleaning solution	Carcinogenic, corrosive, oxidizer, toxic	Replace with suitable commercially available cleanser
Chromomycin A3 (CA3)	Teratogenic, toxic	
Congo red	Mutagenic, teratogenic	See Basic Protocol 2
Coomassie brilliant blue G	Mutagenic	See Basic Protocol 2
Crystal violet		See Basic Protocol 2
Cresyl violet acetate	Mutagenic	See Basic Protocol 2
Cyanides (e.g., KCN, NaCN)	Toxic	Contact with acid will liberate HCN gas; see Basic Protocol 4
Cyanines (e.g., Cy3, Cy5)	Toxic	
Cyanogen bromide (CNBr)	Toxic	See Basic Protocol 4

continued

Table A.2A.1 Commonly Used Hazardous Chemicals[a], *continued*

Chemical	Hazards	Remarks[b]
2′-Deoxycoformycin (dCF, pentostatin)	Teratogenic, toxic	
4′,6-Diamidino-2-phenylindole (DAPI)	Mutagenic	
Diaminobenzidine (DAB)	Carcinogenic	See Basic Protocol 1
1,4-Diazabicyclo[2,2,2]-octane (DABCO)	Toxic	Forms an explosive complex with hydrogen peroxide
Dichloroacetic acid (DCA)	Carcinogenic, corrosive, toxic	
Dichloromethane (methylene chloride)	Carcinogenic, mutagenic, teratogenic, toxic	
Diethylamine (DEA)	Corrosive, flammable liquid, toxic	
Diethylpyrocarbonate (DEPC)	Carcinogenic, toxic	
Diethyl sulfate	Carcinogenic, teratogenic, toxic	See Basic Protocol 5
Diisopropyl fluorophosphate (DFP)	Highly toxic, cholinesterase inhibitor, neurotoxin	See Basic Protocol 11
Dimethyldichlorosilane	Corrosive, flammable liquid, toxic	See Basic Protocol 3
Dimethyl sulfate (DMS)	Carcinogenic, toxic	See Basic Protocol 5
Dimethyl sulfoxide (DMSO)	Flammable liquid, toxic	Enhances absorption through skin
Diphenylamine (DPA)	Teratogenic, toxic	
2,5-Diphenyloxazole (PPO)	Toxic	
Dithiothreitol (DTT)	Toxic	
Eosin B		See Basic Protocol 2
Erythrosin B	Carcinogenic, mutagenic	See Basic Protocol 2
Ether	Flammable liquid, toxic	May form explosive peroxides on standing; do not dry with NaOH or KOH
Ethidium bromide (EB)	Mutagenic, toxic	See Basic Protocol 2 or 6
Ethyl methanesulfonate (EMS)	Carcinogenic, toxic	See Basic Protocol 5
Fluorescein and derivatives	Carcinogenic, toxic	
5-Fluoro-2′-deoxyuridine (FUdR)	Teratogenic, toxic	
Fluoroorotic acid (FOA)	Toxic	
Formaldehyde	Carcinogenic, flammable liquid, teratogenic, toxic	
Formamide	Teratogenic, toxic	
Formic acid	Corrosive, toxic	May explode when heated >180°C in a sealed tube
Glutaraldehyde	Corrosive, teratogenic, toxic	
Guanidinium thiocyanate	Toxic	
Hoechst 33258 dye	Mutagenic, toxic	
Hydrochloric acid, concentrated	Corrosive, teratogenic, toxic	

continued

Table A.2A.1 Commonly Used Hazardous Chemicals[a], *continued*

Chemical	Hazards	Remarks[b]
Hydrogen peroxide (30%)	Carcinogenic, corrosive, mutagenic, oxidizer	Avoid bringing into contact with organic materials, which may form explosive peroxides; may decompose violently in contact with metals, salts, or oxidizable materials; see Basic Protocol 7
Hydroxylamine	Corrosive, flammable, mutagenic, toxic	Explodes in air at >70°C
3-β-Indoleacrylic acid (IAA)	Carcinogenic	
Iodine	Corrosive, toxic	See Basic Protocol 8
Iodoacetamide	Carcinogenic, mutagenic, toxic	
Janus green B	Carcinogenic, mutagenic	See Basic Protocol 2
Lead compounds	Carcinogenic, toxic	
2-Mercaptoethanol (2-ME)	Stench, toxic	
Mercury compounds	Teratogenic, toxic	See Basic Protocol 9
Methionine sulfoximine (MSX)	Teratogenic, toxic	
Methotrexate (amethopterin)	Carcinogenic, mutagenic, teratogenic, toxic	
Methylene blue	Mutagenic, toxic	See Basic Protocol 2
Methyl methanesulfonate (MMS)	Carcinogenic, toxic	See Basic Protocol 5
Mycophenolic acid (MPA)	Teratogenic, toxic	
Neutral red	Mutagenic	See Basic Protocol 2
Nigrosin, water soluble		See Basic Protocol 2
Nitric acid, concentrated	Corrosive, oxidizer, teratogenic, toxic	
Nitroblue tetrazolium (NBT)	Toxic	
Orcein, synthetic		See Basic Protocol 2
Oxonols	Toxic	
Paraformaldehyde	Toxic	
Phenol	Carcinogenic, corrosive, teratogenic, toxic	Readily absorbed through the skin
Phenylmethylsulfonyl fluoride (PMSF)	Enzyme inhibitor	See Basic Protocol 11
Phorbol 12-myristate 13-acetate (PMA)	Carcinogenic, toxic	
Phycoerythrins (PE)	Toxic	
Piperidine	Flammable liquid, teratogenic, toxic	
Potassium hydroxide, concentrated	Corrosive, toxic	Produces a highly exothermic reaction when solid is added to water
Propane sultone	Carcinogenic, toxic	See Basic Protocol 5
Propidium iodide (PI)	Mutagenic	See Basic Protocol 2 or 6
Pyridine	Flammable liquid, toxic	
Rhodamine and derivatives	Toxic	
Rose Bengal	Carcinogenic, teratogenic	See Basic Protocol 2
Safranine O	Mutagenic	See Basic Protocol 2

continued

Table A.2A.1 Commonly Used Hazardous Chemicals[a], *continued*

Chemical	Hazards	Remarks[b]
Sodium azide	Carcinogenic, toxic	Adding acid liberates explosive volatile, toxic hydrazoic acid; can form explosive heavy metal azides, e.g., with plumbing fixtures—*do not* discharge down drain; see Basic Protocol 10
Sodium deoxycholate (Na-DOC)	Carcinogenic, teratogenic, toxic	
Sodium dodecyl sulfate (sodium lauryl sulfate, SDS)	Sensitizing, toxic	
Sodium hydroxide, concentrated	Corrosive, toxic	A highly exothermic reaction ensues when the solid is added to water
Sodium nitrite	Carcinogenic	
Sulfuric acid, concentrated	Corrosive, oxidizer, teratogenic, toxic	Reaction with water is very exothermic; always add concentrated sulfuric acid to water, *never* water to acid
SYTO dyes	Toxic	
Tetramethylammonium chloride (TMAC)	Toxic	
N,N,N′,N′-Tetramethyl-ethylenediamine (TEMED)	Corrosive, flammable liquid, toxic	
Texas Red (sulforhodamine 101, acid chloride)	Toxic	
Toluene	Flammable liquid, teratogenic, toxic	
Toluidine blue O	Mutagenic, toxic	See Basic Protocol 2
Nα-p-Tosyl-L-lysine chloromethyl ketone (TLCK)	Toxic, enzyme inhibitor	See Basic Protocol 11
N-p-Tosyl-L-phenylalanine chloromethyl ketone (TPCK)	Toxic, mutagenic, enzyme inhibitor	See Basic Protocol 11
Trichloroacetic acid (TCA)	Carcinogenic, corrosive, teratogenic, toxic	
Triethanolamine acetate (TEA)	Carcinogenic, toxic	
Trifluoroacetic acid (TFA)	Corrosive, toxic	
Trimethyl phosphate (TMP)	Carcinogenic, mutagenic, teratogenic	May explode on distillation
Trypan blue	Carcinogenic, mutagenic, teratogenic	See Basic Protocol 2
Xylenes	Flammable liquid, teratogenic, toxic	

[a]For extensive information on the hazards of these and other chemicals as well as cautionary details, see Bretherick (1986), O'Neil (2001), Furr (2000), Lewis (1999), Lunn and Sansone (1994a), and Bretherick et al. (1999).

[b]*CAUTION:* These chemicals should be handled only in a chemical fume hood by knowledgeable workers equipped with eye protection, lab coat, and gloves. The laboratory should be equipped with a safety shower and eye wash. Additional protective equipment may be required.

9. Control pest populations. Windows in the lab that can be opened must be equipped with screens to exclude insects.
10. Use furniture that is easy to clean—i.e., with smooth, waterproof surfaces and as few seams as possible.
11. Keep biohazard waste in covered containers free from leaks. Use orange bags or red bags as required by institutional procedure (see discussion under Disposal of Biohazards). Autoclave and dump hazardous waste without undue delay.

Disinfectants for Biohazards

Major laboratory suppliers sell disinfectants based on quaternary ammonium compounds that are acceptable for routine biohazard decontamination (see *SUPPLIERS APPENDIX*). These include Roccal (Baxter), Vesphene II (Fisher), and industrial disinfectants such as concentrated Lysol. 10% chlorine bleach may also be used for decontamination. An antimicrobial liquid soap (e.g., Vionex; Fisher) should be provided in a dispenser near the sink so that no one need handle the outside of the container to use it.

Disposal of Biohazards

Most institutions have defined procedures for disposal of biohazardous waste, but the following are common to all of these systems:

1. All contaminated material should be placed in autoclavable bags, which should be contained in a plastic trash pail or wire frame. If large numbers of disposable pipets or other pointed instruments are being used, it may be necessary to double-bag the material. Autoclavable biohazard bags are sold by all major laboratory supply houses. In some institutions it is necessary to color code the biohazard waste (e.g., orange bags for less dangerous waste and red ones for suspected HIV-containing material). All of these bags are marked with the universal biohazard symbol.
2. At time of disposal, the bags are loosely closed (not completely sealed) with temperature-sensitive autoclave tape (also widely available from supply houses), placed in an autoclavable basin, and sterilized at 121°C. When the tape indicates that sterilization temperature has been achieved, it is then possible to dispose of the waste by ordinary means.
3. At many institutions, contaminated hypodermic needles, scalpels, broken glass, and other sharp objects must be disposed of separately. These must be placed in appropriate "sharps" containers (e.g., Baxter), which may be autoclaved when full.

SAFE USE OF HAZARDOUS CHEMICALS

It is not possible in the space available to list all the precautions required for handling hazardous chemicals. Many texts have been written about laboratory safety (see Literature Cited and Key References). Obviously, all national and local laws should be obeyed, as well as all institutional regulations. Controlled substances are regulated by the Drug Enforcement Administration (*http://www.doj.gov/dea*). By law, Material Safety Data Sheets (MSDSs) must be readily available. All laboratories should have a Chemical Hygiene Plan (29CFR Part 1910.1450); institutional safety officers should be consulted as to its implementation. Help is (or should be) available from your institutional Safety Office; use it.

Chemicals must be stored properly for safety. Certain chemicals cannot be easily or safely mixed with and should not be stored near certain other chemicals, because their reaction is violently exothermic or yields a toxic product. Some examples of incompatibility are

Table A.2A.2 Examples of Chemical Incompatibility

Chemical	Incompatible with
Acetic acid	Aldehydes, bases, carbonates, chromic acid, ethylene glycol, hydroxides, hydroxyl compounds, metals, nitric acid, oxidizers, perchloric acid, peroxides, phosphates, permanganates, xylene
Acetone	Acids, amines, concentrated nitric and sulfuric acid mixtures, oxidizers, plastics
Acetylene	Copper, halogens, mercury, oxidizers, potassium, silver
Alkali metals, alkaline earth metals	Acids, aldehydes, carbon dioxide, carbon tetrachloride or other chlorinated hydrocarbons, halogens, ketones, plastics, sulfur, water
Ammonia (anhydrous)	Acids, aldehydes, amides, calcium hypochlorite, hydrofluoric acid, halogens, heavy metals, mercury, oxidizers, plastics, sulfur
Ammonium nitrate	Acids, alkalis, chlorates, chloride salts, flammable and combustible materials, metals, organic materials, phosphorus, reducing agents, sulfur, urea
Aniline	Acids, aluminum, dibenzoyl peroxide, oxidizers, plastics
Arsenical materials	Any reducing agent
Azides	Acids, heavy metals, oxidizers
Bromine	Acetaldehyde, alcohols, alkalis, amines, ammonia, combustible materials, ethylene, fluorine, hydrogen, ketones (e.g., acetone, carbonyls), metals, petroleum gases, sodium carbide, sulfur
Calcium oxide	Acids, ethanol, fluorine, organic materials, water
Carbon (activated)	Alkali metals, calcium hypochlorite, halogens, oxidizers
Carbon tetrachloride	Sodium
Chlorates	Acids, ammonium salts, finely divided organic or combustible materials, powdered metals, sulfur
Chlorine	Acetylene or other hydrocarbons, alcohols, ammonia, benzene, butadiene, butane, combustible materials, ethylene, flammable compounds (e.g., hydrazine), hydrogen, hydrogen peroxide, iodine, metals, methane, nitrogen, oxygen, propane (or other petroleum gases), sodium carbide, sodium hydroxide
Chlorine dioxide	Ammonia, hydrogen, hydrogen sulfide, mercury, methane, organic materials, phosphine, phosphorus, potassium hydroxide, sulfur
Chromic acid, chromic oxide	Acetic acid, acetone, alcohols, alkalis, ammonia, bases, benzene, camphor, flammable liquids, glycerin (glycerol), hydrocarbons, metals, naphthalene, organic materials, phosphorus, plastics
Copper	Acetylene, calcium, hydrocarbons, hydrogen peroxide, oxidizers
Cumene hydroperoxide	Acids (organic or inorganic)
Cyanides	Acids, alkaloids, aluminum, iodine, oxidizers, strong bases
Flammable liquids	Ammonium nitrate, chromic acid, halogens, hydrogen peroxide, nitric acid, oxidizing agents in general, oxygen, sodium peroxide
Fluorine	All other chemicals

continued

Table A.2A.2 Examples of Chemical Incompatibility, *continued*

Chemical	Incompatible with
Hydrocarbons (liquid or gas)	See flammable liquids
Hydrocyanic acid	Alkali, nitric acid
Hydrofluoric acid	Ammonia, metals, organic materials, plastics, silica (glass, including fiberglass), sodium
Hydrogen peroxide	All organics, most metals or their salts, nitric acid, phosphorus, sodium, sulfuric acid
Hydrogen sulfide	Acetylaldehyde, fuming nitric acid, metals, oxidizers, sodium, strong bases
Hydroperoxide	Reducing agents
Hypochlorites	Acids, activated carbon
Iodine	Acetylaldehyde, acetylene, ammonia, hydrogen, metals, sodium
Mercury	Acetylene, aluminum, amines, ammonia, calcium, fulminic acid, lithium, oxidizers, sodium
Nitric acid	Acids, nitrites, metals, most organics, plastics, sodium, sulfur, sulfuric acid
Nitrites	Acids
Nitroparaffins	Amines, inorganic bases
Oxalic acid	Mercury, oxidizers, silver, sodium chlorite
Oxygen	All flammable and combustible materials, ammonia, carbon monoxide, grease, metals, oil, phosphorus, polymers
Perchloric acid	All organics, bismuth and alloys, dehydrating agents, grease, hydrogen halides, iodides, paper, wood
Peroxides, organic	Acids (organic or mineral), avoid friction, store cold
Phosphorus (white)	Air, alkalis, oxygen, reducing agents
Potassium chlorate	Acids, ammonia, combustible materials, fluorine, hydrocarbons, metals, organic materials, reducing agents, sugars
Potassium perchlorate	Alcohols, combustible materials, fluorine, hydrazine, metals, organic matter, reducing agents, sulfuric acid
Potassium permanganate	Benzaldehyde, ethylene glycol, glycerin, sulfuric acid
Selenides and tellurides	Reducing agents
Silver	Acetylene, ammonium compounds, fulminic acid, oxalic acid, ozonides, peroxyformic acid, tartaric acid
Sodium	Acids, carbon dioxide, carbon tetrachloride, hydrazine, metals, oxidizers, water
Sodium nitrate	Acetic anhydride, acids, metals, organic matter, peroxyformic acid, reducing agents
Sodium peroxide	Acetic anhydride, benzaldehyde, benzene, carbon disulfide, ethyl acetate, ethyl or methyl alcohol, ethylene glycol, furfural, glacial acetic acid, glycerin, hydrogen sulfide, metals, methyl acetate, oxidizers, peroxyformic acid, phosphorus, reducing agents, sugars, water
Sulfides	Acids
Sulfuric acid	Alcohols, bases, chlorates, perchlorates, permanganates of potassium, lithium, sodium, magnesium, calcium

listed in Table A.2A.2. When in doubt, always consult a current MSDS for information on reactivity, handling, and storage. Chemicals should be separated into general hazard classes and stored appropriately. For example, flammable chemicals such as alcohols, ketones, aliphatic and aromatic hydrocarbons, and other materials labeled flammable should be stored in approved flammable storage cabinets, with those also requiring refrigeration being kept in explosion-proof refrigerators. Strong oxidizers must be segregated. Strong acids (e.g., sulfuric, hydrochloric, nitric, perchloric, and hydrofluoric) should be stored in a separate cabinet well removed from strong bases and from flammable organics. An exception is glacial acetic acid, which is both corrosive and flammable, and which must be stored with the flammables.

Facilities should be appropriate for working with hazardous chemicals. In particular, hazardous chemicals should be handled only in chemical fume hoods, not in laminar flow cabinets. The functioning of the fume hoods should be checked periodically. Laboratories should also be equipped with safety showers and eye-wash facilities. Again, this equipment should be tested periodically to ensure that it functions correctly. Other safety equipment may be required depending on the nature of the materials being handled. In addition, researchers should be trained in the proper procedures for handling hazardous chemicals as well as other laboratory operations—e.g., handling of compressed gases, use of cryogenic liquids, operation of high-voltage power supplies, and operation of lasers of all types.

Before starting work, know the physical and chemical hazards of the reagents used. Wear appropriate protective clothing and have a plan for dealing with spills or accidents; coming up with a good plan on the spur of the moment is very difficult. For example, have the appropriate decontaminating or neutralizing agents prepared and close at hand. Small spills can probably be cleaned up by the researcher. In the case of larger spills, the area should be evacuated and help should be sought from those experienced in and equipped for dealing with spills—e.g., the institutional Safety Office.

Protective equipment should include, at a minimum, eye protection, a lab coat, and gloves. In certain circumstances other items of protective equipment may be necessary (e.g., a face shield). Different types of gloves exhibit different resistance properties (Table A.2A.3). No gloves resist all chemicals, and no gloves resist any chemicals indefinitely. Disposable gloves labeled “exam” or “examination” are primarily for protection from biological materials (e.g., viruses, bacteria, feces, blood). They are not designed for and usually have not been tested for resistance to chemicals. Disposable gloves generally offer *extremely* marginal protection from chemical hazards in most cases and should be removed immediately upon contamination before the chemical can pass through. If possible, design handling procedures to eliminate or reduce potential for contamination. Never assume that disposable gloves will offer the same protection or even have the same properties as nondisposables. Select gloves carefully and always look for some evidence that they will protect against the materials being used. Inspect all gloves before every use for possible holes, tears, or weak areas. Never reuse disposable gloves. Clean reusable gloves after each use and dry carefully inside and out. Observe all common-sense precautions—e.g., do not pipet by mouth, keep unauthorized persons away from hazardous chemicals, do not eat or drink in the lab, wear proper clothing in the lab (sandals, open-toed shoes, and shorts are not appropriate).

Order hazardous chemicals only in quantities that are likely to be used in a reasonable time. Buying large quantities at a lower unit cost is no bargain if someone (perhaps you) has to pay to dispose of surplus quantities. Substitute alcohol-filled thermometers for mercury-filled thermometers, which are a hazardous chemical spill waiting to happen.

Table A.2A.3 Chemical Resistance of Commonly Used Gloves[a,b]

Chemical	Neoprene gloves	Latex gloves	Butyl gloves	Nitrile gloves
*Acetaldehyde	VG	G	VG	G
Acetic acid	VG	VG	VG	VG
*Acetone	G	VG	VG	P
Ammonium hydroxide	VG	VG	VG	VG
*Amyl acetate	F	P	F	P
Aniline	G	F	F	P
*Benzaldehyde	F	F	G	G
*Benzene	P	P	P	F
Butyl acetate	G	F	F	P
Butyl alcohol	VG	VG	VG	VG
Carbon disulfide	F	F	F	F
*Carbon tetrachloride	F	P	P	G
*Chlorobenzene	F	P	F	P
*Chloroform	G	P	P	E
Chloronaphthalene	F	P	F	F
Chromic acid (50%)	F	P	F	F
Cyclohexanol	G	F	G	VG
*Dibutyl phthalate	G	P	G	G
Diisobutyl ketone	P	F	G	P
Dimethylformamide	F	F	G	G
Dioctyl phthalate	G	P	F	VG
Epoxy resins, dry	VG	VG	VG	VG
*Ethyl acetate	G	F	G	F
Ethyl alcohol	VG	VG	VG	VG
*Ethyl ether	VG	G	VG	G
*Ethylene dichloride	F	P	F	P
Ethylene glycol	VG	VG	VG	VG
Formaldehyde	VG	VG	VG	VG
Formic acid	VG	VG	VG	VG
Freon 11, 12, 21, 22	G	P	F	G
*Furfural	G	G	G	G
Glycerin	VG	VG	VG	VG
Hexane	F	P	P	G
Hydrochloric acid	VG	G	G	G
Hydrofluoric acid (48%)	VG	G	G	G
Hydrogen peroxide (30%)	G	G	G	G
Ketones	G	VG	VG	P
Lactic acid (85%)	VG	VG	VG	VG
Linseed oil	VG	P	F	VG
Methyl alcohol	VG	VG	VG	VG
Methylamine	F	F	G	G
Methyl bromide	G	F	G	F
*Methyl ethyl ketone	G	G	VG	P
*Methyl isobutylketone	F	F	VG	P
Methyl methacrylate	G	G	VG	F
Monoethanolamine	VG	G	VG	VG

continued

Table A.2A.3 Chemical Resistance of Commonly Used Gloves[a,b], *continued*

Chemical	Neoprene gloves	Latex gloves	Butyl gloves	Nitrile gloves
Morpholine	VG	VG	VG	G
Naphthalene	G	F	F	G
Naphthas, aliphatic	VG	F	F	VG
Naphthas, aromatic	G	P	P	G
*Nitric acid	G	F	F	F
Nitric acid, red and white fuming	P	P	P	P
Nitropropane (95.5%)	F	P	F	F
Oleic acid	VG	F	G	VG
Oxalic acid	VG	VG	VG	VG
Palmitic acid	VG	VG	VG	VG
Perchloric acid (60%)	VG	F	G	G
Perchloroethylene	F	P	P	G
Phenol	VG	F	G	F
Phosphoric acid	VG	G	VG	VG
Potassium hydroxide	VG	VG	VG	VG
Propyl acetate	G	F	G	F
i-Propyl alcohol	VG	VG	VG	VG
n-Propyl alcohol	VG	VG	VG	VG
Sodium hydroxide	VG	VG	VG	VG
Styrene (100%)	P	P	P	F
Sulfuric acid	G	G	G	G
Tetrahydrofuran	P	F	F	F
*Toluene	F	P	P	F
Toluene diisocyanate	F	G	G	F
*Trichloroethylene	F	F	P	G
Triethanolamine	VG	G	G	VG
Tung oil	VG	P	F	VG
Turpentine	G	F	F	VG
*Xylene	P	P	P	F

[a]Performance varies with glove thickness and duration of contact. An asterisk indicates limited use. Abbreviations: VG, very good; G, good; F, fair; P, poor (do not use).

[b]Adapted from the July 8, 1998, version of the DOE OSH Technical Reference Chapter 5 (APPENDIX C at *http://tis.eh.doe.gov/docs/osh_tr/ch5c.html*). For more information also see Forsberg and Keith (1999).

Although any number of chemicals commonly used in laboratories are toxic if used improperly, the toxic properties of a number of reagents require special mention. Chemicals that exhibit carcinogenic, corrosive, flammable, lachrymatory, mutagenic, oxidizing, teratogenic, toxic, or other hazardous properties are listed in Table A.2A.1. Chemicals listed as carcinogenic range from those accepted by expert review groups as causing cancer in humans to those for which only minimal evidence of carcinogenicity exists. No effort has been made to differentiate the carcinogenic potential of the compounds in Table A.2A.1. Oxidizers may react violently with oxidizable material (e.g., hydrocarbons, wood, and cellulose). Before using any of these chemicals, thoroughly investigate all its characteristics. Material Safety Data Sheets are readily available; they list some hazards but vary widely in quality. A number of texts describing hazardous properties are listed at the end of this Appendix (see Literature Cited). In particular, Sax's *Dangerous Properties of Industrial Materials*, 10th ed. (Lewis, 1999) and the *Handbook of Reactive Chemical Hazards*, 6th ed. (Bretherick et al., 1999) give comprehensive listings of known hazardous

properties; however, these texts list only the known properties. Many chemicals, especially fluorochromes, have been tested only partially or not at all. Prudence dictates that, unless there is good reason for believing otherwise, all chemicals should be regarded as volatile, highly toxic, flammable human carcinogens and should be handled with great care.

Waste should be segregated according to institutional requirements, for example, into solid, aqueous, nonchlorinated organic, and chlorinated organic material, and should always be disposed of in accordance with all applicable federal, state, and local regulations. Extensive information and cautionary details along with techniques for the disposal of chemicals in laboratories have been published (Bretherick, 1986; Lunn and Sansone, 1994a; O'Neil, 2001; Furr, 2000). Some commonly used disposal procedures are outlined in Basic Protocols 1 to 11. Incorporation of these procedures into laboratory protocols can help to minimize waste disposal problems. Alternate Protocols 1 to 7 describe decontamination methods for some of the chemicals. Support Protocols 1 to 9 describe analytical techniques that are used to verify that reagents have been decontaminated; with modification, these assays may also be used to determine the concentration of a particular chemical.

DISPOSAL METHODS

A number of procedures for the disposal of hazardous chemicals are available; protocols for the disposal and decontamination of some hazardous chemicals commonly encountered in molecular biology laboratories are listed in Table A.2A.4. These procedures are necessarily brief; for full details consult the original references or a collection of these procedures (see Lunn and Sansone, 1994a).

CAUTION: These disposal methods should be carried out only in a chemical fume hood by workers equipped with eye protection, a lab coat, and gloves. Additional protective equipment may be necessary.

BASIC PROTOCOL 1

DISPOSAL OF BENZIDINE AND DIAMINOBENZIDINE

Benzidine and diaminobenzidine can be degraded by oxidation with potassium permanganate (Castegnaro et al., 1985; Lunn and Sansone, 1991a). This protocol presents a method for decontamination of benzidine and diaminobenzidine in bulk. This method can also be adapted to the decontamination of benzidine and diaminobenzidine spills (see Alternate Protocol 1). These compounds can also be removed from solution using horseradish peroxidase in the presence of hydrogen peroxide (see Alternate Protocol 2). Destruction and decontamination are >99%. Support Protocol 1 is used to test for the presence of benzidine and diaminobenzidine.

Materials

Benzidine or diaminobenzidine tetrahydrochloride dihydrate
0.1 M HCl (for benzidine)
0.2 M potassium permanganate: prepare immediately before use
2 M sulfuric acid
Sodium metabisulfite
10 M potassium hydroxide (KOH)

Additional reagents and equipment for testing for the presence of aromatic amines (see Support Protocol 1)

Table A.2A.4 Protocols for Disposal of Some Hazardous Chemicals

Protocol	Disposal method for
Basic Protocol 1	Benzidine and diaminobenzidine
Alternate Protocol 1	Spills of benzidine and diaminobenzidine
Alternate Protocol 2	Aqueous solutions of benzidine and diaminobenzidine
Support Protocol 1	Analysis for benzidine and diaminobenzidine
Basic Protocol 2	Biological stains
Alternate Protocol 3	Large volumes of dilute biological stains
Support Protocol 2	Analysis for biological stains
Basic Protocol 3	Silanes
Basic Protocol 4	Cyanide and cyanogen bromide
Support Protocol 3	Analysis for cyanide
Basic Protocol 5	Dimethyl sulfate, diethyl sulfate, methyl methanesulfonate, ethyl methanesulfonate, diepoxybutane, 1,3-propane sultone
Support Protocol 4	Analysis for dimethyl sulfate, diethyl sulfate, methyl methanesulfonate, ethyl methanesulfonate, diepoxybutane, 1,3-propane sultone
Basic Protocol 6	Ethidium bromide and propidium iodide
Alternate Protocol 4	Equipment contaminated with ethidium bromide
Alternate Protocol 5	Ethidium bromide in isopropanol containing cesium chloride
Alternate Protocol 6	Ethidium bromide in alcohols
Support Protocol 5	Analysis for ethidium bromide and propidium iodide
Basic Protocol 7	Hydrogen peroxide
Basic Protocol 8	Iodine
Basic Protocol 9	Mercury compounds
Alternate Protocol 7	Waste water containing mercury
Support Protocol 6	Analysis for mercury
Basic Protocol 10	Sodium azide
Support Protocol 7	Analysis for sodium azide
Support Protocol 8	Analysis for nitrite
Basic Protocol 11	Enzyme inhibitors
Support Protocol 9	Analysis for enzyme inhibitors

1. For each 9 mg benzidine, add 10 ml of 0.1 M HCl *or* for each 9 mg diaminobenzidine tetrahydrochloride dihydrate, add 10 ml water. Stir the solution until the aromatic amine has completely dissolved.
2. For each 10 ml of solution, add 5 ml freshly prepared 0.2 M potassium permanganate and 5 ml of 2 M sulfuric acid. Allow the mixture to stand for ≥10 hr.
3. Add sodium metabisulfite until the solution is decolorized.
4. Add 10 M KOH to make the solution strongly basic, pH >12.

   CAUTION: *This reaction is exothermic.*
5. Dilute with 5 vol water and pass through filter paper to remove manganese compounds.
6. Test the filtrate for the presence of aromatic amines (i.e., benzidine or diaminobenzidine; see Support Protocol 1).

7. Neutralize the filtrate with acid and discard.

ALTERNATE PROTOCOL 1

DECONTAMINATION OF SPILLS INVOLVING BENZIDINE AND DIAMINOBENZIDINE

Additional Materials *(also see Basic Protocol 1)*

Glacial acetic acid
1:1 (v/v) 0.2 M potassium permanganate/2 M sulfuric acid: prepare immediately before use

Absorbent material (e.g., paper towels, Kimwipes)
High-efficiency particulate air (HEPA) vacuum (Fisher)

Additional reagents and equipment for testing for the presence of aromatic amines (see Support Protocol 1)

CAUTION: This procedure may damage painted surfaces and Formica.

1. Remove as much of the spill as possible using absorbent material and high-efficiency particulate air (HEPA) vacuuming.
2. Wet the surface with glacial acetic acid until all the amines are dissolved, then add an excess of freshly prepared 1:1 (v/v) 0.2 M potassium permanganate/2 M sulfuric acid to the spill area. Allow the mixture to stand ≥10 hr.
3. Ventilate the area and decolorize with sodium metabisulfite.
4. Mop up the liquid with paper towels. Squeeze the solution out of the towels and collect in a suitable container. Discard towels as hazardous solid waste.
5. Add 10 M KOH to make the solution strongly basic, pH ≥12.

   CAUTION: *This reaction is exothermic.*
6. Dilute with 5 vol water and filter through filter paper to remove manganese compounds.
7. Test the filtrate for the presence of aromatic amines (i.e., benzidine or diaminobenzidine; see Support Protocol 1).
8. Neutralize the filtrate with acid and discard it.
9. Verify complete decontamination by wiping the surface with a paper towel moistened with water and squeezing the liquid out of the towel. Test the liquid for the presence of benzidine or diaminobenzidine (see Support Protocol 1). Repeat steps 1 to 9 as necessary.

ALTERNATE PROTOCOL 2

DECONTAMINATION OF AQUEOUS SOLUTIONS OF BENZIDINE AND DIAMINOBENZIDINE

The enzyme horseradish peroxidase catalyzes the oxidation of the amine to a radical which diffuses into solution and polymerizes. The polymers are insoluble and fall out of solution.

Additional Materials *(also see Basic Protocol 1)*

Aqueous solution of benzidine or diaminobenzidine
1 N HCl or NaOH
3% (v/v) hydrogen peroxide
Horseradish peroxidase (see recipe)
1:1 (v/v) 0.2 M potassium permanganate/2 M sulfuric acid
5% (w/v) ascorbic acid
Porous glass filter *or* Sorvall GLC-1 centrifuge or equivalent

Additional reagents and equipment for testing for the presence of aromatic amines (see Support Protocol 1)

1. Adjust the pH of the aqueous benzidine or diaminobenzidine solution to 5 to 7 with 1 N HCl or NaOH as required and dilute so the concentration of aromatic amines is ≤100 mg/liter.
2. For each liter of solution, add 3 ml of 3% hydrogen peroxide and 300 U horseradish peroxidase. Let the mixture stand 3 hr.
3. Remove the precipitate by filtering the solution through a porous glass filter or by centrifuging 5 min at room temperature in a benchtop centrifuge to pellet the precipitate.

   The precipitate is mutagenic and should be treated as hazardous waste.

4. Immerse the porous glass filter in 1:1 (v/v) 0.2 M potassium permanganate/2 M sulfuric acid. Clean the filter in a conventional fashion and discard potassium permanganate/sulfuric acid solution as described for benzidine and diaminobenzidine (see Basic Protocol 1).
5. For each liter of filtrate, add 100 ml of 5% ascorbic acid.
6. Test the filtrate for the presence of aromatic amines (see Support Protocol 1).
7. Discard the decontaminated filtrate.

ANALYTICAL PROCEDURES TO DETECT BENZIDINE AND DIAMINOBENZIDINE

SUPPORT PROTOCOL 1

Reversed-phase HPLC (Snyder et al., 1997) is used to test for the presence of aromatic amines. The limit of detection is 1 µg/ml for benzidine and 0.25 µg/ml for diaminobenzidine.

Materials

Decontaminated aromatic amine solution

10:30:20 (v/v/v) acetonitrile/methanol/1.5 mM potassium phosphate buffer (1.5 mM K_2HPO_4/1.5 mM KH_2PO_4) (benzidine) *or* 75:25 (v/v) methanol/1.5 mM potassium phosphate buffer (diaminobenzidine)

250-mm × 4.6-mm-i.d. Microsorb C-8 reversed-phase HPLC column (Varian) or equivalent

Additional reagents and equipment for reversed-phase liquid chromatography (Snyder et al., 1997)

Analyze the decontaminated aromatic amine solution by reversed-phase HPLC using a 250-mm × 4.6-mm-i.d. Microsorb C-8 column or equivalent. To detect benzidine, elute with 10:30:20 (v/v/v) acetonitrile/methanol/1.5 mM potassium phosphate buffer at a flow rate of 1.5 ml/min and UV detection at 285 nm. To detect diaminobenzidine, elute with 75:25 (v/v) methanol/1.5 mM potassium phosphate buffer at a flow rate of 1 ml/min and UV detection at 300 nm.

BASIC PROTOCOL 2

DISPOSAL OF BIOLOGICAL STAINS

Biological stains (Table A.2A.5), as well as ethidium bromide and propidium iodide, can be removed from solution using the polymeric resin Amberlite XAD-16. The decontaminated solution may be disposed of as nonhazardous aqueous waste and the resin as hazardous solid waste. The volume of contaminated resin generated is much smaller than the original volume of the solution of biological stain, so the waste disposal problem is greatly reduced. The final concentration of any remaining stain should be less than the limit of detection (see Support Protocol 2 and Table A.2A.5). In each case decontamination should be >99%. This protocol describes a method for batch decontamination in which the resin is stirred in the solution to be decontaminated and removed by filtration at the end of the reaction time. Large volumes of biological stain can be decontaminated using a column (see Alternate Protocol 3). For full details refer to the original literature (Lunn and Sansone, 1991b) or a compilation (Lunn and Sansone, 1994a).

Materials

Amberlite XAD-16 resin (Supelco)
100 µg/ml biological stain in water

Additional reagents and equipment for testing for the presence of biological stain (see Support Protocol 2)

For batch decontamination of 20 ml stain

1a. Add 1 g Amberlite XAD-16 to 20 ml of 100 µg/ml biological stain in water.

Table A.2A.5 Decontamination of Biological Stains

Compound	Time required for complete decontamination	Volume of solution (ml) decontaminated per gram resin
Acridine orange	18 hr	20
Alcian blue 8GX	10 min	500
Alizarin red S	18 hr	5
Azure A	10 min	80
Azure B	10 min	80
Brilliant blue R	2 hr	80
Congo red	2 hr	40
Coomassie brilliant blue G	2 hr	80
Cresyl violet acetate	2 hr	40
Crystal violet	30 min	200
Eosin B	30 min	40
Erythrosin B	18 hr	10
Ethidium bromide	4 hr	20
Janus green B	30 min	80
Methylene blue	30 min	80
Neutral red	10 min	500
Nigrosin	2 hr	80
Orcein	2 hr	200
Propidium iodide	2 hr	20
Rose Bengal	3 hr	20
Safranine O	1 hr	20
Toluidine blue O	30 min	80
Trypan blue	2 hr	40

For aqueous solutions having stain concentrations other than 100 µg/ml, use proportionately greater or lesser amounts of resin to achieve complete decontamination.

For solutions of erythrosin B, use 2 g Amberlite XAD-16 for 20 ml stain.

2a. Stir the mixture for at least the time indicated in Table A.2A.5.

For batch decontamination of larger volumes of stain

1b. Add 1 g Amberlite XAD-16 to the volume of a 100 µg/ml biological stain in water indicated in Table A.2A.5.

2b. Stir the mixture for at least 18 hr.

3. Remove the resin by filtration through filter paper.

4. Test the filtrate for the presence of the biological stain (see Support Protocol 2).

5. Discard the resin as hazardous solid waste and the decontaminated filtrate as liquid waste.

CONTINUOUS-FLOW DECONTAMINATION OF AQUEOUS SOLUTIONS OF BIOLOGICAL STAINS

ALTERNATE PROTOCOL 3

For treating large volumes of dilute aqueous solutions of biological stains (Table A.2A.5), it is possible to put the resin in a column and run the contaminated solution through the column in a continuous-flow system (Lunn et al., 1994). Limited grinding of the Amberlite XAD-16 resin increases its efficiency.

Additional Materials *(also see Basic Protocol 2)*

25 µg/ml biological stain in water
Methanol (optional)

300-mm × 11-mm-i.d. glass chromatography column fitted with threaded adapters and flow-regulating valves at top and bottom nut and insert connectors, and insertion tool (Ace Glass) *or* 300-mm × 15-mm-i.d. glass chromatography column (Spectrum 124010, Fisher)
Glass wool
1.5-mm-i.d. × 0.3-mm-wall Teflon tubing
Waring blender (optional)
Rubber stopper fitted over a pencil
QG 20 lab pump (Fluid Metering)

Additional reagents and equipment for testing for the presence of biological stain (see Support Protocol 2)

Using a slurry of Amberlite XAD-16

1a. Prepare a 300-mm × 11-mm-i.d. glass chromatography column. To prevent clogging of the column outlet, place a small plug of glass wool at the bottom of the chromatography column. Connect 1.5-mm-i.d. × 0.3-mm wall Teflon tubing to the adapters using nut and insert connectors. Attach the tubing using an insertion tool.

2a. Mix 10 g Amberlite XAD-16 and 25 ml water in a beaker and stir 5 min to wet the resin.

Using a finely ground Amberlite XAD-16 slurry

1b. Prepare a 300-mm × 15-mm-i.d. glass chromatography column. To prevent clogging of the column outlet, place a small plug of glass wool at the bottom of the chromatography column.

Table A.2A.6 Breakthrough Volumes for Continuous-Flow Decontamination of Biological Stains

Compound	Breakthrough volume (ml)		
	Limit of detection	1 ppm	5 ppm
Acridine orange	465	>990	>990
Alizarin red S	120	150	240
Azure A	615	810	>975
Azure B	630	882	>1209
Cresyl violet acetate	706	>1396	>1396
Crystal violet	1020	>1630	>1630
Ethidium bromide	260	312	416
Janus green B	170	650	>870
Methylene blue	420	645	1050
Neutral red	>2480	>2480	>2480
Safranine O	365	438	584
Toluidine blue O	353	494	606

2b. Grind 20 g Amberlite XAD-16 with 200 ml water for exactly 10 sec in a Waring blender.

3. Pour the resin slurry into the column through a funnel. As the resin settles, tap the column with a rubber stopper fitted over a pencil to encourage even packing. Attach a QG 20 lab pump.

4. Pump the 25-µg/ml biological stain solution through the column at 2 ml/min.

   Alternatively, gravity flow coupled with periodic adjustment of the flow-regulating valve can be used.

5. Check the effluent from the column for the presence of biological stain (see Support Protocol 2). Stop the pump when stain is detected.

   Table A.2A.6 lists breakthrough volumes at different detection levels for a number of biological stains.

6. Discard the decontaminated effluent and the contaminated resin appropriately.

7. Many biological stains can be washed off the resin with methanol so the resin can be reused. Discard the methanol solution of stain as hazardous organic liquid waste.

SUPPORT PROTOCOL 2

ANALYTICAL PROCEDURES TO DETECT BIOLOGICAL STAIN

Depending on the biological stain, the filtrate or eluate from the decontamination procedure can be analyzed using either UV absorption (A) or fluorescence detection (F).

Materials

Filtrate or eluate from biological stain decontamination (see Basic Protocol 2 or Alternate Protocol 3)
pH 5 buffer (see recipe)
1 M KOH solution
20 µg/ml calf thymus DNA in TBE electrophoresis buffer, pH 8.1 (*APPENDIX 2E*)

Table A.2A.7 Methods for Detecting Biological Stains[a]

Compound	Reagent[b]	Procedure	Wavelength(s)(nm)	Limit of detection (ppm)
Acridine orange	DNA solution	F	ex 492, em 528	0.0032
Alcian blue 8GX		A	615	0.9
Alizarin red S	1 M KOH	A	556	0.46
Azure A		A	633	0.15
Azure B		A	648	0.13
Brilliant blue R		A	585	1.0
Congo red		A	497	0.25
Coomassie brilliant blue G		A	610	1.7
Cresyl violet acetate	pH 5 buffer	F	ex 588, em 618	0.021
Crystal violet		A	588	0.1
Eosin B		A	514	0.21
Erythrosin B		F	ex 488, em 556	0.025
Ethidium bromide		F	ex 540, em 590	0.05
Janus green B		A	660	0.6
Methylene blue		A	661	0.13
Neutral red	pH 5 buffer	A	540	0.6
Nigrosin		A	570	0.8
Orcein	1 M KOH	A	579	1.15
Propidium iodide	DNA solution	F	ex 350, em 600	0.1
Rose Bengal		F	ex 520, em 576	0.04
Safranine O		F	ex 460, em 582	0.03
Toluidine blue O		A	626	0.2
Trypan blue		A	607	0.22

[a]Abbreviations: A, absorbance; em, emission; ex, excitation; F, fluorescence
[b]See Support Protocol 2

Test the filtrate or eluate from the biological stain decontamination procedure using the appropriate method as indicated in Table A.2A.7.

Traces of acid or base on the resin may induce color changes in the stain. Accordingly, with cresyl violet acetate or neutral red, mix aliquots of the filtrate with 1 vol pH 5 buffer before analyzing. With alizarin red S and orcein, mix aliquots of the filtrate with 1 vol of 1 M KOH solution before analyzing.

Increase the fluorescence of solutions of acridine orange, ethidium bromide, and propidium iodide by mixing an aliquot of the filtrate with an equal volume of 20 µg/ml calf thymus DNA in TBE electrophoresis buffer, pH 8.1. Let the solution stand 15 min before measuring the fluorescence.

DISPOSAL OF CHLOROTRIMETHYLSILANE AND DICHLORODIMETHYLSILANE

BASIC PROTOCOL 3

Silane-containing compounds are hydrolyzed to hydrochloric acid and polymeric silicon-containing material (Patnode and Wilcock, 1946).

1. Hydrolyze silane-containing compounds by cautiously adding 5 ml silane to 100 ml vigorously stirred water in a flask. Allow the resulting suspension to settle.
2. Remove any insoluble material by filtration and discard it with the solid or liquid hazardous waste.
3. Neutralize the aqueous layer with base and discard it.

BASIC PROTOCOL 4

DISPOSAL OF CYANIDES AND CYANOGEN BROMIDE

Inorganic cyanides (e.g., NaCN) and cyanogen bromide (CNBr) are oxidized by sodium hypochlorite (NaOCl; e.g., Clorox) in basic solution to the much less toxic cyanate ion (Lunn and Sansone, 1985a). Destruction is >99.7%.

Materials

Cyanide (e.g., NaCN) or cyanogen bromide (CNBr)
1 M NaOH
5.25% (v/v) sodium hypochlorite (NaOCl; i.e., standard household bleach)

Additional reagents and equipment for testing for the presence of cyanide (see Support Protocol 3)

1. Dissolve cyanide in water to give a concentration ≤25 mg/ml *or* dissolve CNBr in water to give a concentration ≤60 mg/ml.

   If necessary, dilute aqueous solutions so the concentration of NaCN or CNBr does not exceed the limit.

2. Mix 1 vol NaCN or CNBr solution with 1 vol 1 M NaOH and 2 vol fresh 5.25% NaOCl. Stir the mixture 3 hr.

   IMPORTANT NOTE: *With age bleach may become ineffective; use of fresh bleach is strongly recommended.*

3. Test the reaction mixture for the presence of cyanide (see Support Protocol 3).

4. Neutralize the reaction mixture and discard it.

SUPPORT PROTOCOL 3

ANALYTICAL PROCEDURE TO DETECT CYANIDE

This protocol is used to detect cyanide or cyanogen bromide at ≥3 µg/ml.

Materials

Cyanide or cyanogen bromide decontamination reaction mixture (see Basic Protocol 4)
Phosphate buffer (see recipe)
10 mg/ml sodium ascorbate in water: prepare fresh daily
100 mg/liter NaCN in water: prepare fresh weekly
10 mg/ml chloramine-T in water: prepare fresh daily
Cyanide detection reagent (see recipe)
Sorvall GLC-1 centrifuge or equivalent

1. If necessary to remove suspended solids, centrifuge two 1-ml aliquots of the cyanide or cyanogen bromide decontamination reaction mixture 5 min in a benchtop centrifuge, room temperature. Add each supernatant to 4 ml phosphate buffer in separate tubes.

2. If an orange or yellow color appears, add 10 mg/ml freshly prepared sodium ascorbate dropwise until the mixture is colorless, but do not add more than 2 ml.

3. Add 200 µl of 100 mg/liter NaCN to one reaction mixture (control solution).

4. Add 1 ml freshly prepared 10 mg/ml chloramine-T to each tube. Shake the tubes and let them stand 1 to 2 min.

5. Add 1 ml cyanide detection reagent, shake, and let stand 5 min.

   A blue color indicates the presence of cyanide. If destruction has been complete and the analytical procedure has been carried out correctly, the treated reaction mixture should be colorless and the control solution, which contains NaCN, should be blue.

6. Centrifuge tubes 5 min, room temperature, if necessary to remove suspended solids. Measure the absorbance at 605 nm with appropriate standards and blanks.

DISPOSAL OF DIMETHYL SULFATE, DIETHYL SULFATE, METHYL METHANESULFONATE, ETHYL METHANESULFONATE, DIEPOXYBUTANE, AND 1,3-PROPANE SULTONE

BASIC PROTOCOL 5

Dimethyl sulfate is hydrolyzed by base to methanol and methyl hydrogen sulfate (Lunn and Sansone, 1985b). Subsequent hydrolysis of methyl hydrogen sulfate to methanol and sulfuric acid is slow. Methyl hydrogen sulfate is nonmutagenic and a very poor alkylating agent. The other compounds can be hydrolyzed with base in a similar fashion (Lunn and Sansone, 1990a). Destruction is >99%. A method to verify that decontamination is complete is also provided (see Support Protocol 4).

NOTE: The reaction times given in the protocol should give good results; however, reaction time may be affected by such factors as the size and shape of the flask and the rate of stirring. The presence of two phases indicates that the reaction is not complete, and stirring should be continued until the reaction mixture is homogeneous.

Materials

Dimethyl sulfate, diethyl sulfate, methyl methanesulfonate, ethyl methanesulfonate, diepoxybutane, or 1,3-propane sultone
5 M NaOH

Additional reagents and equipment for testing for the presence of dimethyl sulfate, diethyl sulfate, methyl methanesulfonate, ethyl methanesulfonate, diepoxybutane, or 1,3-propane sultone (see Support Protocol 4)

For bulk quantities of dimethyl sulfate

1a. Add 100 ml dimethyl sulfate to 1 liter of 5 M NaOH. Stir the reaction mixture.

2a. Fifteen minutes after all the dimethyl sulfate has gone into solution, neutralize the reaction mixture with acid.

For bulk quantities of diethyl sulfate

1b. Add 100 ml diethyl sulfate to 1 liter of 5 M NaOH. Stir the reaction mixture 24 hr.

2b. Neutralize the reaction mixture with acid.

For bulk quantities of methyl methanesulfonate, ethyl methanesulfonate, diepoxybutane, and 1,3-propane sultone

1c. Add 1 ml methyl methanesulfonate, ethyl methanesulfonate, or diepoxybutane, or 1 g of 1,3-propane sultone to 10 ml of 5 M NaOH. Stir the reaction mixture 1 hr for 1,3-propane sultone, 2 hr for methyl methanesulfonate, 22 hr for diepoxybutane, or 24 hr for ethyl methanesulfonate.

2c. Neutralize the reaction mixture with acid.

3. Test the reaction mixture for the presence of the original compound (see Support Protocol 4).

4. Discard the decontaminated reaction mix.

SUPPORT PROTOCOL 4

ANALYTICAL PROCEDURE TO DETECT THE PRESENCE OF DIMETHYL SULFATE, DIETHYL SULFATE, METHYL METHANESULFONATE, ETHYL METHANESULFONATE, DIEPOXYBUTANE, AND 1,3-PROPANE SULTONE

This protocol is used to verify decontamination of solutions containing dimethyl sulfate, diethyl sulfate, methyl methanesulfonate, ethyl methanesulfonate, diepoxybutane, or 1,3-propane sultone. The detection limit for this assay is 40 µg/ml for dimethyl sulfate, 108 µg/ml for diethyl sulfate, 84 µg/ml for methyl methanesulfonate, 1.1 µg/ml for ethyl methanesulfonate, 360 µg/ml for diepoxybutane, and 264 µg/ml for 1,3-propane sultone.

Materials

Reaction mixture containing dimethyl sulfate, diethyl sulfate, methyl methanesulfonate, ethyl methanesulfonate, diepoxybutane, or 1,3-propane sultone
98:2 (v/v) 2-methoxyethanol/acetic acid
5% (w/v) 4-(4-nitrobenzyl)pyridine in 2-methoxyethanol
Piperidine
2-Methoxyethanol

1. Dilute an aliquot of the reaction mixture with 4 vol water.
2. Add 100 µl diluted reaction mixture to 1 ml of 98:2 (v/v) 2-methoxyethanol/acetic acid. Swirl to mix.
3. Add 1 ml of 5% (w/v) 4-(4-nitrobenzyl)pyridine in 2-methoxyethanol. Heat 10 min at 100°C, then cool 5 min in ice.
4. Add 0.5 ml piperidine and 2 ml of 2-methoxyethanol.
5. Measure the absorbance of the violet reaction mixture at 560 nm against an appropriate blank.

   The absorbance of a decontaminated solution should be 0.000.

BASIC PROTOCOL 6

DISPOSAL OF ETHIDIUM BROMIDE AND PROPIDIUM IODIDE

Ethidium bromide and propidium iodide in water and buffer solutions may be degraded by reaction with sodium nitrite and hypophosphorous acid in aqueous solution (Lunn and Sansone, 1987); destruction is >99.87%. This reaction may also be used to decontaminate equipment contaminated with ethidium bromide (see Alternate Protocol 4; Lunn and Sansone, 1989) and to degrade ethidium bromide in organic solvents (see Alternate Protocol 5 and Alternate Protocol 6; Lunn and Sansone, 1990b). Ethidium bromide and propidium iodide may also be removed from solution by adsorption onto Amberlite XAD-16 resin (see Basic Protocol 2).

Materials

Ethidium bromide– or propidium iodide–containing solution in water, buffer, or 1 g/ml cesium chloride
5% (v/v) hypophosphorous acid: prepare fresh daily by diluting commercial 50% reagent 1/10
0.5 M sodium nitrite: prepare fresh daily
Sodium bicarbonate

Additional reagents and equipment for testing for the presence of ethidium bromide or propidium iodide (see Support Protocol 5)

1. If necessary, dilute the ethidium bromide– or propidium iodide–containing solution so the concentration of ethidium bromide or propidium iodide is ≤0.5 mg/ml.

2. For each 100 ml solution, add 20 ml of 5% hypophosphorous acid solution and 12 ml of 0.5 M sodium nitrite. Stir briefly and let stand 20 hr.
3. Neutralize the reaction mixture by adding sodium bicarbonate until the evolution of gas ceases.
4. Test the reaction mixture for the presence of ethidium bromide or propidium iodide (see Support Protocol 5).
5. Discard the decontaminated reaction mixture.

DECONTAMINATION OF EQUIPMENT CONTAMINATED WITH ETHIDIUM BROMIDE

ALTERNATE PROTOCOL 4

Glass, stainless steel, Formica, floor tile, and the filters of transilluminators have been successfully decontaminated using this protocol. No change in the optical properties of the transilluminator filter could be detected, even after a number of decontamination cycles.

Materials

Equipment contaminated with ethidium bromide
Decontamination solution (see recipe)
Sodium bicarbonate

Additional reagents and equipment for testing for the presence of ethidium bromide (see Support Protocol 5)

1. Wash the equipment contaminated with ethidium bromide once with a paper towel soaked in decontamination solution.

   The pH of the decontamination solution is 1.8. If this would be too corrosive for the surface to be decontaminated, wash with a paper towel soaked in water instead.

2. Wash the surface five times with paper towels soaked in water using a fresh towel each time.
3. Soak all the towels 1 hr in decontamination solution.
4. Neutralize the decontamination solution by adding sodium bicarbonate until the evolution of gas ceases.
5. Test the decontamination solution for the presence of ethidium bromide (see Support Protocol 5).
6. Discard the decontamination solution and the paper towels as nonhazardous liquid and solid wastes.

DECONTAMINATION OF ETHIDIUM BROMIDE IN ISOPROPANOL SATURATED WITH CESIUM CHLORIDE

ALTERNATE PROTOCOL 5

Materials

Ethidium bromide in isopropanol saturated with cesium chloride
Decontamination solution (see recipe)
Sodium bicarbonate

Additional reagents and equipment for testing for the presence of ethidium bromide (see Support Protocol 5)

1. If necessary, dilute the ethidium bromide in isopropanol saturated with cesium chloride so the concentration of ethidium bromide is ≤1 mg/ml.
2. For each volume of ethidium bromide solution, add 4 vol decontamination solution. Stir the reaction mixture 20 hr.
3. Neutralize the reaction mixture by adding sodium bicarbonate until the evolution of gas ceases.
4. Test the reaction mixture for the presence of ethidium bromide (see Support Protocol 5).
5. Discard the decontaminated solution.

ALTERNATE PROTOCOL 6

DECONTAMINATION OF ETHIDIUM BROMIDE IN ISOAMYL ALCOHOL AND 1-BUTANOL

Materials

Ethidium bromide in isoamyl alcohol or 1-butanol
Decontamination solution (see recipe)
Activated charcoal
Sodium bicarbonate
Separatory funnel

Additional reagents and equipment for testing for the presence of ethidium bromide

1. If necessary, dilute the ethidium bromide in isoamyl alcohol or 1-butanol so the concentration is ≤1 mg/ml final.
2. For each volume of ethidium bromide solution, add 4 vol decontamination solution. Stir the two-phase reaction mixture rapidly for 72 hr.
3. For each 100 ml of reaction mixture, add 2 g activated charcoal. Stir another 30 min.
4. Filter the reaction mixture.
5. Neutralize the filtrate by adding sodium bicarbonate until the evolution of gas ceases. Separate the layers using a separatory funnel.

   More alcohol may tend to separate from the aqueous layer on standing.
6. Test the alcohol and aqueous layers for the presence of ethidium bromide.
7. Discard the alcohol and aqueous layers appropriately. Discard the activated charcoal as solid waste.

   The aqueous layer contains 4.6% 1-butanol or 2.3% isoamyl alcohol.

SUPPORT PROTOCOL 5

ANALYTICAL PROCEDURE TO DETECT ETHIDIUM BROMIDE OR PROPIDIUM IODIDE

This protocol is used to verify that solutions no longer contain ethidium bromide or propidium iodide. The limits of detection are 0.05 parts per million (ppm) for ethidium bromide and 0.1 ppm for propidium iodide.

Materials

Reaction mixture containing ethidium bromide or propidium iodide
TBE buffer, pH 8.1 (*APPENDIX 2E*)
20 µg/ml calf thymus DNA in TBE buffer, pH 8.1

1. Mix 100 µl reaction mixture containing ethidium bromide or propidium iodide with 900 µl TBE buffer, pH 8.1.

2. Add 1 ml of 20 µg/ml calf thymus DNA in TBE, pH 8.1. Prepare a blank solution (100 µl water + 900 µl TBE + 1 ml of 20 µg/ml calf thymus DNA) and control solutions containing known quantities of ethidium bromide or propidium iodide. Let the mixtures stand 15 min.
3. To detect ethidium bromide, measure the fluorescence with an excitation wavelength of 540 nm and an emission wavelength of 590 nm. To detect propidium iodide, measure the fluorescence with an excitation wavelength of 350 nm and an emission wavelength of 600 nm.

   If a spectrophotofluorometer is not available, fluorescence of ethidium bromide can be qualitatively determined using a hand-held UV lamp on the long-wavelength setting (Lunn and Sansone, 1991c).

DISPOSAL OF HYDROGEN PEROXIDE

BASIC PROTOCOL 7

Hydrogen peroxide can be reduced with sodium metabisulfite (Lunn and Sansone, 1994b).

Materials

30% hydrogen peroxide
10% (w/v) sodium metabisulfite
10% (w/v) potassium iodide
1 M HCl
1% (w/v) starch indicator solution

1. Add 5 ml of 30% hydrogen peroxide to 100 ml of 10% sodium metabisulfite. Stir the mixture at room temperature until the temperature starts to drop, indicating that the reaction is over.
2. Test for the presence of hydrogen peroxide by adding a few drops of the reaction mixture to an equal volume of 10% potassium iodide. Add a few drops of 1 M HCl to acidify the reaction mixture, then add a drop of 1% starch indicator solution.

   A deep blue color indicates the presence of excess oxidant. If necessary, add more 10% sodium metabisulfite until the starch test is negative.
3. Discard the decontaminated mixture.

REDUCTION OF IODINE

BASIC PROTOCOL 8

Iodine is reduced with sodium metabisulfite to iodide (Lunn and Sansone, 1994b).

Materials

Iodine
10% (w/v) sodium metabisulfite
1 M HCl
1% (w/v) starch indicator solution

1. Add 5 g iodine to 100 ml of 10% sodium metabisulfite. Stir the mixture until the iodine has completely dissolved.
2. Acidify a few drops of the reaction mixture by adding a few drops of 1 M HCl. Add 1 drop of 1% starch indicator solution.

   A deep blue color indicates the presence of iodine. If reduction is not complete, add more sodium metabisulfite solution.
3. Dispose of the decontaminated solution.

BASIC PROTOCOL 9

DISPOSAL OF MERCURY COMPOUNDS

Solutions of mercuric acetate can be decontaminated using Dowex 50X8-100, a strongly acidic gel-type ion-exchange resin with a sulfonic acid functionality. Solutions of mercuric chloride can be decontaminated using Amberlite IRA-400(Cl), a strongly basic gel-type ion-exchange resin with a quaternary ammonium functionality. The final concentration of mercury is <3.8 ppm (Lunn and Sansone, 1994a). On a small scale it is most convenient to stir the resin in the solution to be decontaminated, but on a larger scale, or for routine use, it may be more convenient to pass the solution through a column packed with the resin. Although the volume of waste that must be disposed of is greatly reduced using this technique, a small amount of waste (i.e., the resin contaminated with mercury) remains and must be discarded appropriately. Resin can be regenerated by washing with acid, but the concentrated metal-containing solution generated by this must also be disposed of appropriately. Mercury may also be removed from laboratory waste water using a column of iron powder (see Alternate Protocol 7). Support Protocol 6 is used to detect the presence of mercury.

Materials

Solution containing ≤1600 ppm mercuric acetate *or* ≤1350 ppm mercuric chloride
Dowex 50X8-100 ion-exchange resin *or* Amberlite IRA-400(Cl) ion-exchange resin

Additional reagents and equipment to test for the presence of mercury (see Support Protocol 6)

1a. *For mercuric acetate:* For each 200 ml of solution containing ≤1600 ppm mercuric acetate, add 1 g Dowex 50X8-100 ion-exchange resin. Stir the mixture 1 hr, then filter through filter paper.

1b. *For mercuric chloride:* For each 200 ml of solution containing ≤1350 ppm mercuric chloride, add 1 g Amberlite IRA-400(Cl) ion-exchange resin. Stir the mixture 6 hr, then filter through filter paper.

The speed and efficiency of decontamination will depend on factors such as the size and shape of the flask and the rate of stirring.

3. Test the filtrate for the presence of mercury (see Support Protocol 6).

4. Discard the decontaminated filtrate and the mercury-containing resin appropriately.

ALTERNATE PROTOCOL 7

DECONTAMINATION OF WASTE WATER CONTAINING MERCURY

Laboratory waste water that contains mercury is decontaminated by passing it through a column of iron powder. The mercury forms mercury amalgam and stays on the column. Some metallic mercury remains in solution but this can be removed by aeration. The final concentration of mercury is <5 ppb (Shirakashi et al., 1986).

Materials

Iron powder, 60 mesh
Waste water containing ≤2.5 ppm mercury
6-mm-i.d. column

1. Pack a 6-mm-i.d. column with 1 g of 60-mesh iron powder.

Use a fresh column for each treatment.

2. Pass ≤2 liters of water containing ≤2.5 ppm of mercury through the column at a flow rate of 250 ml/hr.

Solutions containing a higher concentration of mercury may also be treated, but this will result in a higher final concentration of mercury (e.g., treating a 100-ppm solution in this fashion yielded 33 ppb final).

Some iron ends up in solution and can be removed by adjusting the pH to 8. The resulting precipitated $Fe(OH)_3$ can then be removed by filtration.

3. Aerate the resulting effluent to remove traces of metallic mercury and continue aeration 30 min after the last of the effluent has emerged from the column. Vent the metallic mercury removed from the solution by aeration into the fume hood or capture it in a mercury trap.

   The effluent contains <5 ppb mercury. The presence of iodide or polypeptone may necessitate several treatments to reduce the mercury to an acceptable level.

ANALYTICAL PROCEDURE TO DETECT MERCURY

SUPPORT PROTOCOL 6

Atomic absorption spectroscopy with detection at 253.7 nm, a slit width of 0.7 nm, and a limit of detection of 3.8 ppm can be used to determine the concentration of mercury in solution for experiments involving ion-exchange resins. A Hiranuma mercury meter model HG-1 can be used for experiments involving iron powder.

DISPOSAL OF SODIUM AZIDE

BASIC PROTOCOL 10

Sodium azide can be oxidized by ceric ammonium nitrate (Manufacturing Chemists Association, 1973) to nitrogen (Mason, 1967) or by nitrous acid (National Research Council, 1983) to nitrous oxide (Mason, 1967); destruction is >99.996%. Sodium azide in buffer solution may also be degraded by the addition of sodium nitrite (Lunn and Sansone, 1994a). The reaction proceeds much more readily at low pH, but if sufficient sodium nitrite is added, it will proceed to completion even at high pH. At low pH, it may be possible to completely degrade the azide present in the buffer with less than the amount of sodium nitrite indicated. However, the reaction mixture must be carefully checked to ensure that no azide remains (see Support Protocol 7). At high pH it is possible for unreacted azide to remain in the presence of excess nitrite. Residual nitrite can be detected using Support Protocol 8.

CAUTION: Some toxic nitrogen dioxide may be produced as a by-product of these reactions, so they should always be carried out in a chemical fume hood.

Materials

Sodium azide or solution containing sodium azide
Ceric ammonium nitrate
10% (w/v) potassium iodide
1 M HCl
1% (w/v) starch indicator solution
Sodium nitrite
4 M sulfuric acid

Additional reagents and equipment to test for the presence of sodium azide (see Support Protocol 7) *or* nitrite (see Support Protocol 8)

Decontamination using ceric ammonium nitrate

1a. For each gram of sodium azide, add 9 g ceric ammonium nitrate to 30 ml of water, and stir until it has dissolved.

2a. Dissolve each gram of sodium azide in 5 ml water. Add this solution to the ceric ammonium nitrate solution at the rate of 1 ml each min. Stir 1 hr.

   If the reaction is carried out on a larger scale, an ice bath may be required for cooling.

3a. Check that the reaction is still oxidizing by adding a few drops of the reaction mixture to an equal volume of 10% potassium iodide. Acidify the mixture with 1 drop 1 M HCl and add 1 drop 1% starch indicator solution.

The deep blue color of the starch-iodine complex indicates that excess oxidant is present. If excess oxidant is not present, add more ceric ammonium nitrate.

4a. Test for the presence of sodium azide (see Support Protocol 8).

5a. Discard the decontaminated reaction mixture.

Decontamination using sodium nitrite

1b. For each 5 g sodium azide, dissolve 7.5 g sodium nitrite in 30 ml water.

2b. Dissolve each 5 g sodium azide in 100 ml water. Add the sodium nitrite solution with stirring. Slowly add 4 M sulfuric acid until the reaction mixture is acidic to litmus. Stir 1 hr.

CAUTION: *It is important to add the sodium nitrite, then the sulfuric acid. Adding these reagents in reverse order will generate explosive, volatile, toxic hydrazoic acid.*

If the reaction is carried out on a large scale, an ice bath may be required for cooling.

3b. Check that there is excess nitrous acid in the reaction. Add a few drops of the reaction mixture to an equal volume of 10% potassium iodide. Acidify the mixture with 1 drop 1 M HCl. Add 1 drop starch indicator solution.

The deep blue color of the starch-iodine complex indicates that excess nitrous acid is present. If excess nitrous acid is not present, add more sodium nitrite.

4b. If excess nitrous acid is present, test for the presence of sodium azide (see Support Protocol 7).

5b. Discard the decontaminated reaction mixture.

Decontamination of sodium azide in buffer

1c. If necessary, dilute the buffer solution with water so the concentration of sodium azide is ≤1 mg/ml.

2c. For each 50 ml buffer solution add 5 g sodium nitrite. Stir the reaction 18 hr.

3c. Test for the presence of sodium azide (see Support Protocol 7).

4c. Discard the decontaminated reaction solution.

SUPPORT PROTOCOL 7

ANALYTICAL PROCEDURES TO DETECT SODIUM AZIDE

Sodium azide is analyzed by reacting azide ion with 3,5-dinitrobenzoyl chloride to form 3,5-dinitrobenzoyl azide, which can be detected by reversed-phase HPLC. The limit of detection of this assay is 0.2 µg/ml sodium azide. This protocol works only in the absence of nitrite; verify that all of the nitrite has been destroyed by sulfamic acid by using the method detailed later in this Appendix (see Support Protocol 8).

Materials

Reaction mixture from sodium azide treated with ceric ammonium nitrate or sodium nitrite
1 M KOH
Acetonitrile
Sodium azide indicator solution (see recipe)
0.2 M HCl

20% (w/v) sulfamic acid
3,5-dinitrobenzoyl chloride
50:50 (v/v) acetonitrile/water

Sorvall GLC-1 centrifuge or equivalent
25-cm × 4.6-mm-i.d. Microsorb C-8 reversed-phase HPLC column (Varian) or equivalent

Additional reagents and equipment for reversed-phase liquid chromatography (Snyder et al., 1997)

To analyze for azide in the presence of ceric salts

1a. To a 10-ml aliquot of the reaction mixture from sodium azide treated with ceric ammonium nitrate add 40 ml water. Add 5 ml of this diluted solution to 3 ml of 1 M KOH and mix by shaking.

If <3 ml of 1 M KOH is used, precipitation of ceric salts will not be complete.

2a. Centrifuge the mixture 5 min, room temperature.

3a. Remove 2 ml supernatant and add to 1 ml acetonitrile. Add 1 drop sodium azide indicator solution, add 0.2 M HCl dropwise until the mixture turns yellow, then add 1 drop more.

To analyze for azide in the presence of nitrite

1b. To 5 ml of the reaction mixture from sodium azide treated with sodium nitrite add ≥1 ml sulfamic acid to remove excess nitrite. Let stand ≥3 min.

More sulfamic acid solution may be required for strongly basic reaction mixtures or those containing high concentrations of nitrite. Complete removal of nitrite can be checked by using a modified Griess reagent (see Support Protocol 8).

At high pH the reaction between azide and nitrite is quite slow, so the presence of excess nitrite does not mean that all the azide has been degraded.

2b. Add 1 drop sodium azide indicator solution, then basify the mixture by adding 1 M KOH until it turns purple (typically, 3 to 10 ml are required).

3b. Add 2 ml acetonitrile. Add 0.2 M HCl dropwise until the mixture turns yellow, then add 1 drop more.

If >1 ml sulfamic acid is used, add 4 ml acetonitrile.

4. Prepare a 10% (w/v) solution of 3,5-dinitrobenzoyl chloride in acetonitrile.

5. Add 50 µl of 10% dinitrobenzoyl chloride/acetonitrile to the reaction mix (step 3a or 3b). Shake the mixture and let it stand ≥3 min.

Longer standing times have no effect on the HPLC analysis. However, it is crucial to use freshly prepared 3,5-dinitrobenzoyl chloride solution within minutes of its preparation. It is generally most convenient to prepare all the analytical samples with the fresh solution at the beginning of the day and analyze them over the course of the day.

6. Analyze 20 µl of each reaction mixture by reversed-phase HPLC (Snyder et al., 1997) using a mobile phase of 50:50 (v/v) acetonitrile/water with a flow rate of 1 ml/min and UV detection at 254 nm.

The peak for 3,5-dinitrobenzoyl azide elutes at ~9 min.

SUPPORT PROTOCOL 8

ANALYTICAL PROCEDURE TO DETECT NITRITE

This protocol uses a modified Griess reagent to test for the presence of nitrite. The limit of detection of this assay is 0.06 μg/ml nitrite. A similar procedure uses *N*-(1-naphthyl)-ethylenediamine (Cunniff, 1995).

Materials

α-Naphthylamine
15% (v/v) aqueous acetic acid
Sulfanilic acid solution (see recipe)
Reaction mixture treated to remove excess nitrite (see Support Protocol 7, step 1b)

1. Prepare the modified Griess reagent by boiling 0.1 g α-naphthylamine in 20 ml water until it dissolves. While the solution is still hot, pour it into 150 ml of 15% aqueous acetic acid. Add 150 ml sulfanilic acid solution.

   This reagent should be stored at room temperature in a brown bottle.

   CAUTION: *α-Naphthylamine is a carcinogen.*

2. Add 3 ml of the reaction mixture treated to remove excess nitrite to 1 ml modified Griess reagent. Let stand 6 min at room temperature.

3. Measure the absorbance at 520 nm against a suitable blank.

BASIC PROTOCOL 11

DISPOSAL OF ENZYME INHIBITORS

The enzyme inhibitors *p*-amidinophenylmethanesulfonyl fluoride (APMSF), 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), phenylmethylsulfonyl fluoride (PMSF; Lunn and Sansone, 1994c), diisopropyl fluorophosphate (DFP; Lunn and Sansone, 1994d), *N*α-*p*-tosyl-L-lysine chloromethyl ketone (TLCK), and *N*-*p*-tosyl-L-phenylalanine chloromethyl ketone (TPCK; Lunn and Sansone, 1994c) may be degraded by reaction with 1 M NaOH. Destruction is >99.8% (except TPCK >98.3%). The exact reaction conditions depend on the solvent (see Table A.2A.8). The solutions that were decontaminated are representative of those described in the literature.

Materials

Solutions of APMSF, AEBSF, PMSF, DFP, TLCK, or TPCK in buffer, DMSO, isopropanol, or water
1 M NaOH
Glacial acetic acid

Additional reagents and equipment for testing for the presence of the enzyme inhibitors (see Support Protocol 9)

1. If necessary, dilute the solutions with the same solvent so that the concentrations given in Table A.2A.8 are not exceeded.

   Bulk quantities of AEBSF, PMSF, and TPCK may be dissolved in isopropanol and bulk quantities of APMSF and TLCK may be dissolved in water at the concentrations shown in Table A.2A.8. Bulk quantities of DFP (a liquid) may be mixed directly with 1 M NaOH, taking care to make sure that all the DFP has mixed thoroughly, in the ratio shown in Table A.2A.8 (e.g., 40 μl DFP with 1 ml of 1 M NaOH).

2. Add 1 M NaOH so that the ratio of solution to 1 M NaOH is that listed in Table A.2A.8.

3. Shake to ensure complete mixing, check that the solution is strongly basic (pH ≥12), and allow to stand for the time given in Table A.2A.8.

Table A.2A.8 Conditions for the Destruction of Enzyme Inhibitors

Compound	Concentration	Solvent	Solution: 1 M NaOH	Time
AEBSF	1 mM	Buffer(pH 5.0-8.0)	1:0.1	1 hr
AEBSF	20 mM	DMSO	1:10	24 hr
AEBSF	20 mM	Isopropanol	1:10	24 hr
APMSF	2.5 mM	Buffer(pH 5.0-8.0)	1:0.1	1 hr
APMSF	25 mM	DMSO	1:5	24 hr
APMSF	25 mM	50:50 isopropanol:pH 3 buffer	1:5	24 hr
APMSF	100 mM	Water	1:5	24 hr
DFP	10 mM	Buffer (pH 6.4-7.2)	1:0.2	18 hr
DFP	200 mM	DMF	1:2	18 hr
DFP	pure	—	1:25	1 hr
DFP	10 mM	Water	1:0.2	18 hr
PMSF	10 mM	Buffer (pH 5.0-8.0)	1:0.1	1 hr
PMSF	100 mM	DMSO	1:5	24 hr
PMSF	100 mM	Isopropanol	1:5	24 hr
TLCK	1 mM	Buffer (pH 5.0-8.0)	1:0.1	18 hr
TLCK	5 mM	DMSO	1:5	18 hr
TLCK	5 mM	Water	1:0.1	18 hr
TPCK	1 mM	Buffer (pH 5.0-8.0)	1:0.1	18 hr
TPCK	1 mM	DMSO	1:0.1	18 hr
TPCK	1 mM	Isopropanol	1:0.1	18 hr

4. Neutralize the reaction mixture with acetic acid and test for the presence of residual enzyme inhibitor (see Support Protocol 9).
5. Discard the decontaminated reaction mixture.

ANALYTICAL PROCEDURES TO DETECT ENZYME INHIBITORS

SUPPORT PROTOCOL 9

DFP can be determined using a complex procedure involving the inhibition of chymotrypsin activity. For more information, refer to Lunn and Sansone (1994d). A gas chromatographic method has also been described by Degenhardt-Langelaan and Kientz (1996). AEBSF, APMSF, PMSF, TLCK, and TPCK may be determined by reversed-phase HPLC (Snyder et al., 1997). The chromatographic conditions and limits of detection are shown in Table A.2A.9 (Lunn and Sansone, 1994c).

Materials

Decontaminated enzyme inhibitor solutions
Acetonitrile (HPLC grade)
Water (HPLC grade)
0.1% (v/v) trifluoroacetic acid in water
10 mM phosphate buffer, pH 7
250-mm × 4.6 mm-i.d. Microsorb C-8 reversed-phase HPLC column (Varian) or equivalent

Additional reagents and equipment for reversed-phase liquid chromatography (Snyder et al., 1997)

Table A.2A.9 HPLC Conditions for Enzyme Inhibitors

Compound	Mobile phase	Detector	Retention time	Limit of detection
AEBSF	40:60 (v/v) acetonitrile:0.1% trifluoroacetic acid	UV 225 nm	9.5 min	0.1 µg/ml
APMSF	40:60 (v/v) acetonitrile:0.1% trifluoroacetic acid	UV 232 nm	7.7 min	0.5 µg/ml
PMSF	50:50 (v/v) acetonitrile:water	UV 220 nm	8 min	0.9 µg/ml
TLCK	40:60 (v/v) acetonitrile:0.1% trifluoroacetic acid	UV 228 nm	9.5 min	0.37 µg/ml
TPCK	48:52 (v/v) acetonitrile:10 mM pH 7 phosphate buffer	UV 228 nm	10.5 min	2 µg/ml

Analyze the decontaminated enzyme inhibitor solutions by reversed-phase HPLC using a 250-mm × 4.6-mm-i.d. Microsorb C-8 reversed-phase column, or equivalent, using the conditions shown in Table A.2A.9. In each case, the injection volume was 20 µl, the separation occurred at ambient temperature, and the flow rate was 1 ml/min. Check the analytical procedures by spiking an aliquot of the acidified reaction mixture with a small quantity of a dilute solution of the compound of interest.

REAGENTS AND SOLUTIONS

*Use deionized, distilled water in all recipes and protocol steps. For common stock solutions, see **APPENDIX 2E**; for suppliers, see **SUPPLIERS APPENDIX**.*

Cyanide detection reagent

Stir 3.0 g barbituric acid in 10 ml water. Add 15 ml of 4-methylpyridine and 3 ml concentrated HCl while continuing to stir. Cool and dilute to 50 ml with water. Store at room temperature.

CAUTION: *This reaction is exothermic.*

Decontamination solution

Dissolve 4.2 g sodium nitrite (0.2 M final) and 20 ml hypophosphorous acid (3.3% w/v final) in 300 ml water. Prepare fresh.

Horseradish peroxidase

Dissolve hydrogen-peroxide oxidoreductase (EC 1.11.1.7 [Type II]; specific activity 150 to 200 purpurogallin U/mg, Sigma) in 1 g/liter sodium acetate to give 30 U/ml. Prepare fresh daily.

For small-scale reactions, a more dilute solution can be used to avoid working with inconveniently small volumes.

pH 5 buffer

2.04 g potassium hydrogen phthalate (0.05 M final)
38 ml 0.1 M potassium hydroxide (15 mM)
H_2O to 200 ml
Store at room temperature

Phosphate buffer

13.6 g monobasic potassium phosphate (KH_2PO_4; 0.1 M final)
0.28 g dibasic sodium phosphate (Na_2HPO_4; 2 mM final)
3.0 g potassium bromide (KBr; 25 mM final)
1 liter H_2O
Store at room temperature

Potassium bromide is necessary to make the assay for cyanide work correctly.

Sodium azide indicator solution

0.1 g bromocresol purple (0.4% final)
18.5 ml 0.01 M potassium hydroxide (KOH; 7.4 mM final)
H_2O to 25 ml
Store at room temperature

Sulfanilic acid solution

Dissolve 0.5 g sulfanilic acid in 150 ml of 15% (v/v) aqueous acetic acid. Use immediately.

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INTERNET RESOURCES

http://www.ilpi.com/msds/index.html

Where to find MSDSs on the internet. Contains links to general sites, government and nonprofit sites, chemical manufacturers and suppliers, pesticides, and miscellaneous sites.

http://www.OSHA.gov

OSHA web site.

http://www.osha-slc.gov/OshStd_data/1910_1450.html

Text of OSHA Standard 29 CFR 1910.1450: Occupational Exposure to Hazardous Chemicals in Laboratories.

http://www.osha-slc.gov/OshStd_data/1910_1000_TABLE_Z-1.html

Tables of permissible exposure limits (PELs) for air contaminants.

http://www.osha-slc.gov/OshStd_data/1910_1000_TABLE_Z-2.html

Tables of PELs for toxic and hazardous substances.

http://hazard.com/msds/index.php

Main site for Vermont SIRI. One of the best general sites to start a search. Browse manufacturers alphabetically (for sheets not in the SIRI collection) or do a keyword search in the SIRI MSDS database. Lots of additional safety links and information.

http://siri.uvm.edu/msds

Alternate site for Vermont SIRI.

http://tis.eh.doe.gov/docs/osh_tr/ch5.html

DOE OSH technical reference chapter on personal protective equipment.

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