

Table 1

## Methods of fixation and extraction for immunofluorescent microscopy

| coagulant  |                                   |  |                       |
|--|-----------------------------------|--|-----------------------|
| Fixative/Extractor   |                                   | Description  | References            |
| MeOH   |                                   | PBS(rapid rinse), MeOH(-20 C, 5min), PBS( 5min)  | [8, 9]                |
| EtOH   |                                   | PBS(rapid rinse), EtOH(-20 C, 5min), PBS( 5min)  | [10]                  |
| Acetone  |                                   | PBS(rapid rinse), Acetone(-20 C, 7min), PBS( 5min)   | [11]                  |
| 10% TCA  |                                   | TCA(ice-cold, 15min), 30mM glycine/PBS(wash x3)  | [10, 12]              |
| 5%Sulfosalicylic acid  |                                   | PBS(rapid rinse), 5%Sulfosalicylic acid(-20 C, 5min), PBS( 5min)   | This paper. Ref. [47] |
| Carnoy (EtOH/CHCl <sub>3</sub> /AcOH, 60/30/10, v/v)   |                                   | No detail information is available, but in the same way of 5%Sulfosalicylic acid   | [55]                  |
| Bouin (saturated picric acid water/formalin/AcOH, 15/5/1, v/v)                                     |                                   | No detail information is available, but in the same way of 5%Sulfosalicylic acid   | [55]                  |
| non-coagulant (Some of the methods are combination of both coagulant and non-coagulant fixatives.) |                                   |  |                       |
| Fixative   | Extractor                         | Descriptions   | References            |
| 3.5% FA/PBS  | acetone                           | 3.5% FA/PBS(rm, 20min), PBS(wash), acetone(-10 C, 7min), air dry   | [52]                  |
| 3.7% FA/PBS  | 0.1% Triton X-100/PBS             | 3.7% FA/PBS(5min), 0.1% Triton X-100/PBS (2min)  | [48]                  |
| 3.7% FA/PBS  | 0.2% Triton X-100/PBS             | 3.7% FA/PBS(20min), 0.2% Triton X-100/PBS (5min)   | [58]                  |
| 3.7% FA/PBS  | 0.5% Triton X-100/PBS             | 3.7% FA/PBS(5min), 0.5% Triton X-100/PBS (5min)  | [9]                   |
| 3.7% FA/PBS  | 1% Triton X-100/PBS               | 3.7% FA/PBS(5min), 1% Triton X-100/PBS (5min)  | [9]                   |
| 3.7% FA/PBS(+)   | 0.2% Triton X-100/PBS             | 3.7% FA/PBS(+)(rm, 20min), 0.2% Triton X-100/PBS (rm, 1min), PBS(rinse, x3)  | [51]                  |
| 4% PFA/PBS   | -                                 | 4% PFA/PBS(rm, 5min), PBS(rinse), NaBH <sub>4</sub> /PBS(0.5mg/ml, rm, 10min), 5% serum/PBS(rm, 60min)   | [45]                  |
| 4% PFA+1%GA/PBS  | 0.2% TX100/PBS                    | 4% PFA+1%GA/PBS(rm, 10min), 0.2% TX100/PBS(rm, 5min), NaBH <sub>4</sub> /PBS(0.5mg/ml, rm, 10min)  | [16]                  |
| 4% PFA/PBS(+)  | 0.1% TX100/PBS                    | 4% PFA/PBS(+)(15min), 50mM NH <sub>4</sub> Cl/PBS(10min), 0.1% TX100/PBS(10min)  | [57]                  |
| 4% PFA/PBS   | 0.2% TX100/PBS                    | 4% PFA(rm, 10min), 0.2% TX100/PBS(rm, 5min), NaBH <sub>4</sub> /PBS(0.5mg/ml, rm, 10min)   | [16, 56]              |
| 4% PFA/HBSS  | acetone                           | 4% PFA/HBSS(37 C, 15min), HBSS(rm, 5min), 100mMglycine/HBSS (rm, 5min), PBS(rm, 5min), acetone(-20 C, 5min), PBS(rm, 5min)   | [8]                   |
| 4% PFA/PBS   | 0.2% TX100+3.6%PFA/PBS<br>acetone | 4% PFA/PBS(rm, 20min), 0.2% TX100+3.6%PFA/PBS(rm, 10min), PBS(rm, 10min), 50%, 100%, 50%acetone(4 C, 3min, each), PBS(rm, 10min), IgG(2mg/ml, rm, 15min)             | [49]                  |
| 4% PFA+0.05% GA/PB   | -                                 | 4% PFA+0.05% GA/PB(4 C, 20min), 100mM PB(wash), 200mM glycine/PB (20min), NaBH <sub>4</sub> (10min, x2), 2%serum/200mM glycine/PB(rm, 30min)                         | [44]                  |
| PLP  | 1% saponin+3% BSA/PBS             | PLP(rm, 10min), 1% saponin+3% BSA/PBS(rm, 10min)   | [17, 53]              |
| LP   | 1% saponin+3% BSA/PBS             | LP(rm, 10min), 1% saponin+3% BSA/PBS(rm, 10min)  | [20]                  |
| -  | 0.2% TX100/MTSB+2.5mM GTP         | MTSB+2.5mM GTP(rm, 30s), 0.2% TX100/MTSB+2.5mM GTP (rm, 30s, x2), 1%GA/MTSB+2.5mM GTP(rm, 10min), NaBH <sub>4</sub> /PBS (0.5mg/ml, rm, 4min, x2), PBS(rm, wash, x3) | [1]                   |
| (3%Formalin+0.05%GA+0.05%TX100/MTPB)   |                                   | 3%Formalin+0.05%GA+0.05%TX100/MTPB(20min), 0.2% TX100/50mM Tris-HCl, pH7.5+150mM NaCl  | [48]                  |
| (3.7% Formalin/PEM/0.2% TX100), MeOH   |                                   | 3.7% Formalin/PEM/0.2% TX100 (5min, rm), PBS(wash), MeOH (ice-cold)  | [59]                  |
| DSP/HBSS   | DSP/Tsb                           | DSP/HBSS(37 C, 10min), DSP/Tsb(37 C, 10min and 5min), 4% PFA/MTSB(37 C, 15min), PBS(rm, 5min), 100mMglycine/PBS (rm, 5min), PBS(rm, 5min)                            | [8]                   |
| 4% PFA/PBS   | 0.1% saponin+5% serum/PBS         | 4% PFA/PBS(30min), 100mM glycine/PBS, 0.1% saponin+5% serum/PBS(20min)   | [54]                  |
| 4% PFA/PBS   | 0.5% NP-40/PBS                    | 4% PFA/PBS(10min), PBS(wash), 0.5% NP-40/PBS(15min)  | [46]                  |
| 4% PFA+320mM sucrose/CB  | 0.5% TX100/CB                     | 4% PFA+320mM sucrose/CB(20min), CB(wash, x3), 0.5%TX100/CB(10min)  | [50]                  |
| (MeOH, 3.7% FA, acetone)   |                                   | TBS-Tx(wash), 2%serum/TBS-Tx(1hour)<br>MeOH(-10 C, 30s), 3.7% FA/PBS (5min), PBS(wash, 15min x2), acetone(-10 C, 5min), PBS(wash)                                    | [60]                  |
| (0.5% DOTMAC+1% PFA/PBS(+))  |                                   | 0.5% DOTMAC+1% PFA/PBS(4 C, 5min), 1% PFA/PBS(4 C, 20min), PBS(rm, 5min, x3), 1% BSA/PBS(rm, 60min)  | This paper.           |

MeOH, methanol; EtOH, ethanol; TCA, trichloroacetic acid.; AcOH, acetic acid; FA, formaldehyde; PFA, paraformaldehyde; GA, glutaraldehyde; DOTMAC, dodecyltrimethylammonium chloride  
 PB, phosphate buffer; PBS, phosphate buffered saline(calcium and magnesium free); PBS(+); PBS containing 1mM CaCl<sub>2</sub> and 1mM MgCl<sub>2</sub>; TX100, Triton X-100; PLP, (2% PFA, 10mM periodate, 75mM lysine, 37.5mM PB, pH7.4); LP, ( 2% PFA, 100mM lysine, 50mM PB, pH7.4, rm); BSA, bovine serum albumin; PHEM, (60mM PIPES, 25mM HEPES, 10mM EGTA, 2mM MgCl<sub>2</sub>, pH6.9); PEM, (100mM PIPES, 5mM EGTA, 2mM MgCl<sub>2</sub>, pH6.8); DSP, dithiobis(succinimidylpropionate); Tsb, 0.5% Triton X-100 in MTSB; MTSB, microtubule stabilizing buffer (1mM EGTA, 4% PEG8000, 100 mM PIPES, pH6.9);  
 MTPB, microtubule-protecting buffer (60mM PIPES, 25mM HEPES, 10mM EGTA, 10mM MgCl<sub>2</sub>, pH6.9); CB, cytoskeleton buffer (10mM MES, 138mM KCl, 3mM MgCl<sub>2</sub>, 2mM EGTA, pH6.1);  
 TBS-Tx, (0.1% Triton X-100, 20mM Tris-HCl, 150mM NaCl, pH7.2).