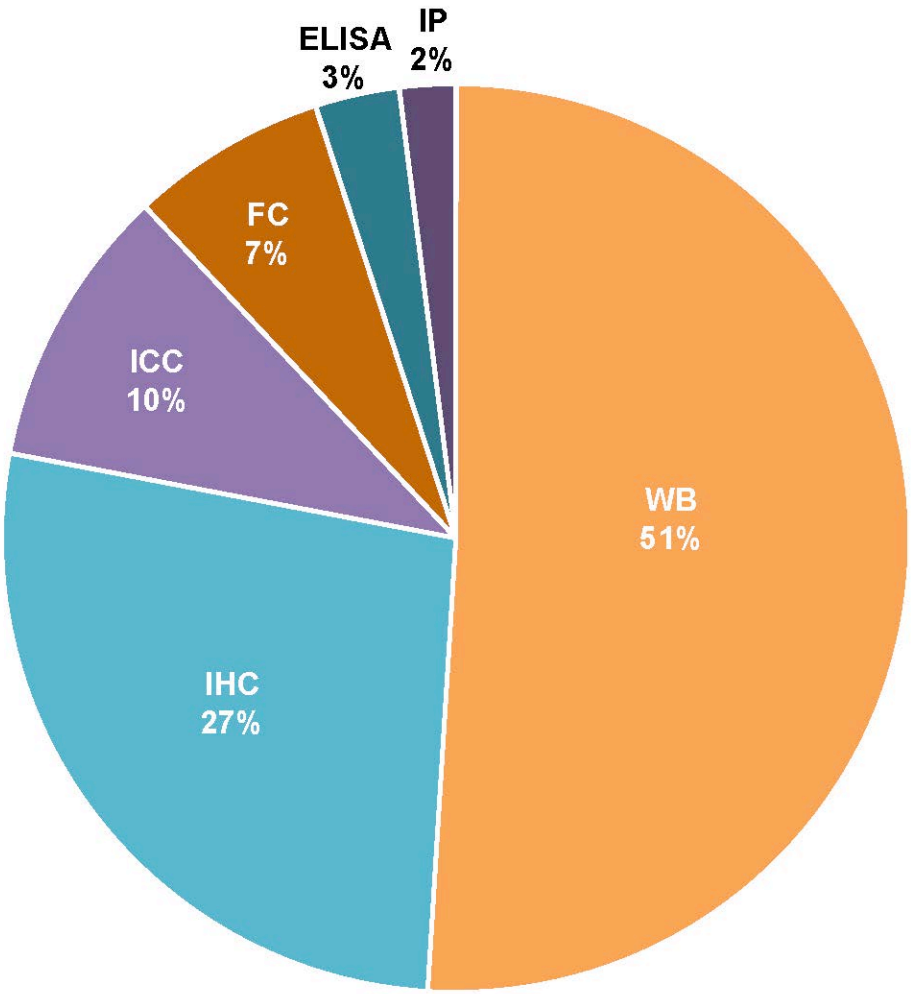

Supplementary Figure 1

Utilization of publicly available antibodies in different applications.

The fraction of publicly available antibodies toward human protein targets is shown with data for the following applications: Western blotting (WB), immunohistochemistry (IHC), immunocytochemistry (ICC), flow cytometry (FC), immunosorbent assays (ELISA), and immunoprecipitation (IP). The numbers are based on information from users and providers reported to the resource portal Antibodypedia[7].

Supplementary Table 1. Protein handling and conditions in various applications

Antibody application	Protein handling (typical)	Description
Western blotting	Denaturing conditions	Proteins are treated with detergent (sodium dodecyl sulfate) but might be partially refolded when blotted onto filter
Immunohistochemistry	Denaturing conditions	Proteins are often cross-linked with formalin and treated with high temperatures (such as 115 degrees Celsius) at elevated pressures (epitope retrieval)
Immunocytochemistry	Denaturing conditions	Cellular proteins are normally fixed and often permeabilization is needed. Organic solvents (such as alcohols and acetone) or cross-linking reagents (such as paraformaldehyde) are typically used
Immunoprecipitation (IP) and chromatin IP	Native conditions	Lysis buffers are often used to stabilize native protein conformation, inhibit protease activity, and allow the protein to be released from the cell or tissue
Sandwich assays	Native conditions	Blood samples (plasma and serum) are normally not pre-treated with denaturing agents, whereas tissue samples are normally treated with lysis buffers
Flow cytometry	Native conditions	Cells are normally not pre-treated with denaturing agents when extracellular markers are used, but permeabilization might be used for intracellular markers
Reverse phase protein arrays	Native conditions	Blood samples (plasma and serum) are normally not pre-treated with denaturing agents, whereas tissue samples are often treated with lysis buffers

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Supplementary Table 2. Additional methods used for general characterization of antibodies

Type of characterization	Description	Method used
Affinity measurements	Determines binding parameters of an antibody for its target	Surface plasmon resonance
Epitope mapping	Determines the site on the target protein where the antibody binds	Peptide arrays, display, alanine scans
Isotype determination	Characterizes an antibody by Fc region of heavy chain	Immunoassay
Sequence determination	Determines the DNA sequence of the complementary region of an antibody	DNA sequencing techniques
Adsorption	Antibody is pre-treated with antigen before assay	Immunohistochemistry
Array-based specificity	Specific binding versus cross-reactivity	Protein or peptide array

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Supplementary Table 3. Relevant references for antibody use and validation

Category	Authors	Description	PMID
References for Antibody Validation and Reproducibility			
Antibody Reproducibility	Bandrowski *et al.,* 2016	Discusses an initiative (RRIDs) to improve reproducibility through unique identifiers for antibodies, model organisms, and tools that are machine-readable and stable	26599696
	Bjorling *et al.,* 2008	Describes a database that assigns publicly available antibodies a validation score based on experimental evidence in an application-specific manner	18667413
	Bordeaux *et al.,* 2010	A review on antibody validation, highlighting the common pitfalls when working with antibodies, common practices for validating antibodies, and levels of commercial antibody validation	20359301
	Bourbellion *et al.,* 2010	Addresses the need for a formal set of standards to report affinity binder reagents and improve reproducibility (MIAPAR)	20622827
	Vasilevsky *et al.,* 2013	Raises the issue of researchers failing to properly reference the affinity reagents used in an experiment (ie, the catalog number and antibody supplier name)	24032093
Western blotting	Gilda *et al.,* 2015	Discusses the technical variability observed for antibodies used in Western blotting and proposes establishment of a reporting standard	26287535
Immunohistochemistry	Deutsch *et al.,* 2008	Describes guidelines for minimum level of information to be reported in gene expression localization experiments (MISFISHIE)	18327244
	Hewitt *et al.,* 2014	Summarizes the necessary controls for validating immunohistochemical data and offers a standard of practice for using IHC in research and diagnostic investigations	25023613
	Howat *et al.,* 2014	Guidelines for a stepwise approach to validate antibodies for immunohistochemical assays	24525140
	O'Hurley *et al.,* 2014	Evaluates challenges faced by the use of immunohistochemical biomarkers and the strategies available for validation of antibodies	24725481
	Ramos-Vara *et al.,* 2014	Highlights technical aspects of IHC and the importance of the antibody-antigen specificity	24129895

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	Schuster *et al.,* 2012	Combines Western blotting and IHC to validate the specificity of 32 antibodies used in research, and found 19 that showed reliable specificity	22393911
Immunocytochemistry	Holmseth *et al.,* 2012	Addresses the ramifications of certain assay controls that may interfere with accurate testing	22215633
	MIACA v.080404	Draft outlining standards for cell-based functional assays	N/A
	Stadler *et al.,* 2012	Describes a platform that combines RNA interference (RNAi) and immunofluorescence to validate antibody binding and subcellular localization of a protein	22361696
Flow cytometry	Lee *et al.,* 2008	MIFlowCyt is a set of standards describing the minimum requirements for the reporting of flow cytometry experiments	18752282
Immunoprecipitation	Marcon *et al.,* 2015	Describes the standard operating procedure for an MS-based approach to measure antibody quality for IP and includes detailed protocols	26121405
Chromatin immunoprecipitation	Landt *et al.,* 2012	Describes how the ENCODE and modENCODE consortia can address antibody validation, experimental replication, sequencing depth, data and metadata reporting, and data quality assessment when analyzing ChIP-seq data	22955991
	ENCODE	The ENCODE website includes antibody characterization standards for transcription factors (updated May 2016) and chromatin remodelers and modifiers (updated Aug 2015)	https://www.encodeproject.org/about/experiment-guidelines/
Reverse phase protein arrays	Jeong *et al.,* 2012	Describes a platform using a full-length protein microarray to screen for monoclonal antibodies with the highest specificity	22307071
	Sjöberg et al., 2016	Validation of antibodies for use in protein microarrays	26417875
RNA interference and genome editing	Olds and Li, 2016	Describes the use of siRNAs to validate antibodies	26998240
Mass spectrometry	MIARE Reporting Checklist v0.8.0	A guide to ensure data reporting of RNAi experiments is publicly accessible	N/A

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	Martinez-Bartolome *et al.,* 2013	Discusses the current approaches and guidelines for reporting quantitative MS	23500130
	MIAPE-Quant v1.0	Outlines the minimum information required to report the use of quantification techniques in proteomics experiments	N/A
IMS	Marcon *et al.,* 2015	Describes the standard operating procedure for an MS-based approach to measure antibody quality for IP	26121405
	MIAPE-MS v2.98	Outlines the minimum information required to report the use of MS in proteomics experiments	N/A
References for Technical and Supporting Information			
Western blotting	Mahmood *et al*., 2012	Considerations for designing Western blot experiments, including the technique, theory, and troubleshooting approaches	23050259
Immunohistochemistry	Schacht *et al.,* 2015	Describes the basic protocol for performing immunohistochemistry with an emphasis on dermatology	25666678
Immunocytochemistry	Burry *et al.,* 2011	Describes a new classification of immunocytochemical controls	20852036
	Waters *et al.,* 2009	Guidelines on digital image acquisition during quantitative fluorescent microscopy	19564400
Flow cytometry	Hulspas *et al.,* 2009	Discusses the major causes of background readings in flow cytometric measurements and the approaches that can improve reliability	19575390
	Kalina *et al.,* 2012	Flow cytometer setting standards and immunophenotyping protocols determined by the EU-supported EuroFlow Consortium	22948490
Immunoprecipitation	Marcon *et al.,* 2015	Describes the standard operating procedure for an MS-based approach to measure antibody quality for IP and includes detailed protocols	26121405
Sandwich assays	Cox *et al.,* 2012	Provides an essential overview of the design principles for implanting robust and automation-friendly assays in immunoassay formats	NBK92434
Reverse phase protein arrays	Akbani *et al.,* 2014	A gathering of insights from three RPPA workshops highlighting current knowledge and future directions	24777629

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Genome editing	Singh et al., 2015	A review on usage of the CRISPR-Cas9 system for the study of mouse genetics	25271304
Mass spectrometry	Abbatiello *et al.,* 2015	Large-scale inter-laboratory study applying multiplexed quantitative peptide assays to detect proteins in biological samples	25693799
	Faupel-Badger *et al.,* 2010	Describes the use of liquid chromatography (LC)-MS/MS as a more accurate method of protein quantification than sandwich assays	20056650
	Liebler *et al.,* 2013	Discusses the sensitivity, precision, and quantitation of multiple-reaction monitoring MS and its applications	23517332
	Martinez-Bartolome *et al.,* 2013	Discusses the current approaches and guidelines for reporting quantitative MS	23500130
	Peterson *et al.,* 2012	Describes parallel reaction monitoring as an improved alternative targeted proteomics approach over selected reaction monitoring	22865924
	Picotti *et al.,* 2012	Selected reaction monitoring as a reliable platform for assessing proteins across multiple samples	22669653
IMS	Edfors *et al.,* 2014	Application of immuno-SILAC for quantification of proteins in complex samples – based on polyclonal antibodies and stable isotope-labeled recombinant protein fragments prior to MS analysis	24722731
	Marcon *et al.,* 2015	Describes the standard operating procedure for an MS-based approach to measure antibody quality for IP	26121405
Proximity assays	Assarsson et al., 2014	Describes use of proximity extension assays in multiplex protein quantification assays and includes protocols	24755770
	Ebai et al., 2015	Describes procedures for developing solid phase proximity ligation assays	25559104
	Lundberg *et al.,* 2011	Study combines proximity extension assay with antibody specificity to detect low-abundance proteins in plasma samples	21646338

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