abcam

Antibody dilutions and titer

Find out which antibody dilution is right for your experiment

Which dilution to use

The rate of binding between antibody and antigen affinity constant can be affected by temperature, pH and buffer constituents. Varying the relative concentrations of an antibody and an antigen solution can also control the extent of antibody-antigen complex formation. As it is not usually possible to change the concentration of the antigen, the optimal working concentration of each individual antibody must be determined with dilutions for each application and set of experimental conditions.

Many of our antibodies have recommended dilutions for various applications included on the datasheet. However, they may require some optimization.

Optimizing the antibody dilution: titration experiments

The optimal antibody concentration, which gives the best staining with minimum background, must be determined experimentally for each assay and is usually determined by using a series of dilutions in a titration experiment. For example, if a product datasheet suggests using a 1:200 dilution, it is recommended to make dilutions of 1:50, 1:100, 1:200, 1:400 and 1:500.

A titration experiment is done by first selecting a fixed incubation time and then a series of experimental dilutions of the antibody. Each dilution should be tested on the same type of sample in order to keep the same experimental conditions.

Many antibodies will have similar batch-to-batch consistency, therefore in most cases only one titration experiment is required. However, especially for polyclonal antibodies, when there is a change in the results of the staining between batches of the same antibody, we recommend performing another titration experiment.

Suggested dilutions for antibodies with no recommended dilution on the datasheet

Unpurified antibody preparations differ significantly in antibody concentration. If the specific antibody concentration of a given unpurified antibody preparation is unknown, we recommend to use a concentration/purification kit and refer to the table below as a guideline.

This table provides various dilutions to use in each application from different sources of antibody:

	Tissue culture supernatant	Ascites	Whole antiserum	Purified antibody
WB/dot blot	1/100	1/1000	1/500	1 µg/mL
IHC/ICC	Neat –1/10	1/100	1/50–1/100	5 µg/mL
EIA/ELISA	1/1000	1/10000	1/500	0.1 µg/mL
FACS/Flow cytometry	1/100	1/1000	1/500	1 µg/mL
IP	-	1/100	1/50–1/100	1–10 µg/mL
Approximate IgG concentration estimate	1–3 mg/mL	5–10 mg/mL	1–10 mg/mL	-