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Matching antibodies to applications

A quick start guide

Immunoassays are versatile and powerful techniques to investigate various protein properties in cells or tissues. Choosing the right antibodies depends on your particular application.

Step 1: application

What are your goals for this experiment? Do you want to see where your protein localizes, or are you more interested in which samples express your protein? This can help you decide which technique is most suitable.

What are you studying?						
Localization		**Concentration**		**Interaction**	**Expression**	**Identification**
Then consider using						
IHC	**ICC**	**Western blot**	**ELISA**	**ELISA**	**Flow cytometry and FACS**	**ELISPOT**
-Low to medium throughput -Fixed cells and tissues -Cellular localization -High spatial resolution -Typically focus on a single protein -Image multiple proteins at once -Qualitative and semi-quantitative	-Low to high throughput -Fixed cells and tissues -Subcellular localization -Spatial resolution limited by tissue structure -Image multiple proteins at once -Qualitative and semi-quantitative	-Low to medium throughput -Cell and tissue lysates -Relative protein levels -Establish molecular weight, isoforms and post-translational modifications -Semi-quantitative	-Medium to high throughput (96 well plate) -Serum/ plasma, live or fixed cells and tissue -Accurate protein levels -Quantitative and qualitative	-Medium to high throughput (96 well plate) -Serum/ plasma, cell or tissue -Molecular interactions -Quantitative and qualitative	-Low to high throughput (up to 384 well plate) -Fixed or live cells -Chemical and physical properties: size, expression of surface markers, etc -Quantify and sort cells (FACS) -Quantitative	-Medium to high throughput (96 well plate) -Live cells -Identification of specific cell(s) secreting analyte of interest -High sensitivity -Quantitative and qualitative

IHC = immunohistochemistry, ICC = immunocytochemistry, FACS = fluorescence-activated cell sorting

Learn more about antibody methods and techniques

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Step 2: primary antibody

With your application decided, you next need to consider the type of primary antibody.

Monoclonal vs polyclonal

Polyclonal antibodies	Monoclonal antibodies
✓ Recognize multiple epitopes on any one antigen ✓ Have a high affinity and can help amplify the signal from low expression proteins ✓ Are more tolerant to minor changes to the antigen ✓ Can be used to detect proteins from species other than that of the immunogen o Prone to batch-batch-variability o Can produce background signal	✓ Recognize only one epitope on the antigen ✓ Generate less background as they are less likely to cross-react with other proteins ✓ Homogeneity is very high and so batch-to-batch variability is low ✓ Extremely efficient at binding an antigen within a mixture of related molecules (e.g. in affinity purification) o May be too specific and therefore less likely to work in range of species o Vulnerable to epitope loss through chemical treatment of the antigen

RabMAb® antibodies

- ✓ Combines the benefits of both monoclonal and polyclonal technology to create highly specific and sensitive rabbit monoclonal antibodies
- ✓ Can be developed recombinantly to provide all the benefits of RabMAb® antibodies, with
  - ✓ The largest commercially available collection of recombinant antibodies
  - ✓ A wider epitope recognition during antibody development, due to a rabbit's lower immune dominance and larger B-cell repertoire
  - ✓ Validation in key applications (WB, IHC, ICC/IF, IP, flow cytometry) and species (human, mouse, rat)
  - ✓ Affinity purified to remove any impurities which otherwise may lead to non-specific signal

Learn more about the advantages of RabMAb® antibody technology

Validation

Confidence that your antibody is binding to the intended target is paramount for valid, reproducible research. That's why we carry out activity, stability and performance checks at our laboratories.

We have also begun to use knockout (KO)-based validation as a standard level of validation for our antibodies. Starting at 400 antibodies each year, we will look to continue this as a part of our rigorous validation process. KO validation ensures that the antibody specific is confirmed using a true negative control.

Read why KO-validated antibodies are so important

Species selection

When choosing your primary antibody, it's important that the species of your sample is different to the species the antibody was raised in (find out why in our antibody guide). Applications such as western blotting using a cell lysate that doesn't contain any endogenous immunoglobulin (IgG), or experiments using primary conjugated antibodies, don't have to worry about this. If you have you work on the same species as your antibody (eg mouse-on-mouse), we can help you to modify your staining protocol.

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Step 3: secondary antibody

The type of secondary antibody you use will often be dependent on the application you're using. Below are some suggested secondary antibodies for the main immunostaining applications.

Secondary antibodies				
IHC	**ICC**	**Western blot**	**ELISA and ELISPOT**	**Flow cytometry and FACS**
-HRP or AP conjugated -Fragment -Biotinylated (avidin/ streptavidin conjugated to enzyme or fluorochomre) -Fluorochromes: Alexa Fluor®, Cy® dyes, FITC, PE -Pre-adsorbed	-Fragment antibodies -Biotinylated (avidin/ streptavidin conjugated to fluorochrome) -Fluorochromes: Alexa Fluor®, Cy® dyes, FITC, PE -Pre-adsorbed	-HRP or AP conjugated -Biotinylated (avidin/ streptavidin conjugated to enzyme) -Fluorochromes: Alexa Flour® (Alexa 680 and Alexa 790) -Light chain specific antibodies/VeriBlot for IP	-HRP or AP conjugated -Biotinylated (avidin/ streptavidin conjugated to enzyme) -Monoclonal (especially subtype specific antibodies such as anti-IgG1)	-Fluorochromes: Alexa Fluor®, Cy® dyes, FITC, PE -Biotinylated (avidin/ streptavidin conjugated to fluorochrome) -Monoclonal -F(ab')$_2$ fragment -Conjugated primary antibodies for maximizing time efficiency

IHC = immunohistochemistry, ICC = immunocytochemistry, FACS = fluorescence-activated cell sorting, HRP = horseradish peroxidase, AP = alkaline phosphatase, IP = immunoprecipitation

Remember

- Select the brightest fluorochromes to label your protein with the lowest expression
- All secondary antibodies should come from the same host species for multiple labeling
- Using serum to block from the same host species of the secondary will significantly reduce background.
- Pre-adsorbed secondary antibodies are useful for multi-color analysis to ensure low cross species reactivity
- Fragment antibodies are smaller and penetrate tissues more efficiently (useful for IHC)
- Biotin conjugates offer enhanced signal amplification

You should now be ready to start your experiments.

Have a look at all of our secondary antibodies