Detection Antibody

1. What are the types of antibodies?

Antibodies can be categorized based on product type and production method as follows:

- Based on Product Types

Primary Antibodies:

- Bind directly to the target antigen.
- Primarily used for detecting and analyzing specific antigens in experiments.

Secondary Antibodies:

- Bind to primary antibodies.
- Often labeled with enzymes, fluorescent dyes, or other markers to facilitate detection or signal amplification.

- Based on Production Methods

Monoclonal Antibodies (mAb):

- Produced by a single B-cell clone.
- Recognize a specific antigen epitope.
- Known for high specificity and consistency.

Polyclonal Antibodies (pAb):

- Produced by multiple B-cell clones.
- Capable of recognizing multiple epitopes of an antigen.
- Typically offer higher sensitivity.

Genetically Engineered Antibodies:

- Produced using genetic engineering techniques.
- Affinity, specificity, or stability can be customized.

2. How are antibodies transported?

Antibodies need to be transported under low-temperature conditions, typically using ice packs or dry ice.

3. How should antibodies be stored and aliquoted?

Storage: Please store under low-temperature conditions. It is recommended to strictly follow the storage conditions specified in the product's Certificate of Analysis (COA). When stored under the recommended conditions, the product can remain stable for at least one year.

Note: Repeated freeze-thaw cycles may cause a decrease or loss of antibody activity.

Aliquoting: To prevent the solution from adhering to the tube walls or cap, centrifuge before use (10,000 x g, 20 seconds). **The size of the aliquots should be determined based on the amount of antibody typically used in experiments. Aliquots should not be less than 10 µL, as smaller volumes are more prone to changes in concentration due to evaporation or adsorption of the antibody to the surface of the vial.**

4. Common Components of Antibody Buffer and Their Functions:

Glycerol: Glycerol can lower the freezing point. By adjusting the final concentration to 50%, antibodies can be stored in liquid form at -20°C, preventing repeated freeze-thaw cycles.

Azide: Azide prevents microbial growth, thereby extending the storage life of antibodies. However, it is toxic and not recommended for use in live-cell experiments, as it may inhibit the activity of certain enzymes.

BSA (Bovine Serum Albumin): As a carrier protein, BSA prevents antibodies from adsorbing onto the walls of storage containers, increasing their stability. However, it may cause nonspecific background signals in some sensitive experiments and may compete with labeling agents in labeling experiments.

5. How to determine the dilution/concentration of a primary antibody?

It's recommended to refer to recommended dilutions/concentrations provided in instruction manual.

If no recommended dilution is provided, refer to an initial dilution chart and adjust based on experimental results.

	Culture supernatant	Ascites	Whole antiserum	Purified antibody
WB/DB	1/100	1/1,000	1/500	1 μg/mL
FC	1/100	1/1,000	1/500	1 μg/mL
ELISA	1/1,000	1/10,000	1/500	0.1 μg/mL
IHC/ICC	Stock solution-1/10	1/100	1/50 - 1/100	5 μg/mL
IP	---	1/100	1/50 - 1/100	1 -10 μg/mL

*Note: The dilution/concentration is provided as a recommended starting point.

6. Has the antibody been validated in other species/applications?

All species and applications in which the antibody has been validated are listed in the datasheet.

7. Can antibodies be detected in untested species?

Even with a high degree of sequence alignment, it cannot be guaranteed that antibodies will function properly in untested species due to the involvement of multiple variables.

8. Will the antibody cross-react with other subtypes or proteins in the same family?

If cross-reactivity data is available, it will be shown in the "Specificity" and "Cross-Reactivity" sections of the datasheet. If no cross-reactivity data is provided, it is recommended to check the alignment of the immunogen sequence with the sequence of the subtype or other proteins of interest.

For alignment scores above 85%, cross-reactivity is possible. For scores significantly below 85%, cross-reactivity is unlikely.

9. What does clone number mean?

A clone number represents a specific cell line cloned from ascites fluid, which is used for antibody production. Since monoclonal antibodies are produced by multiple hosts and cell lines, each cloned cell line is assigned a unique clone number for identification.

10. How to select an isotype control?

Isotype controls are used to confirm that the binding of the primary antibody is specific and not due to other protein interactions or nonspecific Fc receptor binding. The isotype control antibody should match the host species, isotype, and conjugate of the primary antibody. For example, if your primary antibody is an HRP-labeled rat IgG1, the isotype control should also be an HRP-labeled rat IgG1.

11. How to choose a secondary antibody?

Chose the secondary antibody based on the host species of the primary antibody.

For instance, if you are using a rat monoclonal antibody as the primary antibody, you need to select an anti-rat secondary antibody.

12. In Western Blotting (WB), how to choose a loading control (internal reference)?

Due to their stable expression levels in most cases, commonly used loading controls in Western Blot experiments include anti-β-tubulin, anti-β-actin, or anti-GAPDH antibodies. When selecting an appropriate loading control, the following two factors need to be considered:

Type of cell lysate: Choose a loading control that is present in the lysate. For example, since tubulin and actin are located in the cytoplasm, the corresponding antibodies are not suitable for Western Blot experiments using nuclear lysates (in this case, histone antibodies should be selected).

Molecular weight: The molecular weight of the selected loading control protein should differ from that of the target protein to avoid interference between the two bands. For instance, if the molecular weight of the target protein is approximately 39 kDa, it is better to choose tubulin antibody rather than actin antibody as the loading control, because the molecular weights of these two proteins are 50 kDa and 42 kDa, respectively.