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User Guide for Antibodies

1 Classification of Antibodies

A Product type

a Primary Antibody
b Secondary Antibody

B Production process

a Monoclonal Antibody (mAb)
b Polyclonal Antibody (pAb)
c Genetically Engineered Antibody

2 Handling of Antibodies before Opening

It is recommended that before opening the cap, centrifuge the tube (approximately 10,000 × g) for 20-30 seconds before use to prevent the antibody solution from sticking to the walls or cap of the tube. MCE ensures that the total amount of antibody in each tube is the same as mentioned in the level.

3 Shipping Conditions for Antibodies

MCE antibodies are shipped with ice packs. All MCE antibodies are tested for stability before shipment. It is guaranteed that the antibody quality will not be affected by the ambient temperature during regular shipment.

4 Dispensing and Storage of Antibodies for Efficient Use

It is recommended to dispense the antibody into aliquots before storage. Repeated freezing and thawing will reduce the binding capacity of antibodies.

a. Short-term Storage
Most antibodies are stable for 1-2 weeks at 4°C. It is recommended that antibody working solution should be prepared fresh for use.

b. Long-term Storage
Most antibodies are stable at -20°C for 1 year. It is not recommended to store antibodies at -80°C.

c. Enzyme-conjugated antibodies and IgG3 isotype control antibodies should be stored at 4°C to avoid freezing.

d. Conjugated antibodies need to be stored in light-protected environment.

Note: It is highly recommended that the volume of the aliquot should be the amount used for a single experiment and no less than 10 μL.

5 Common Components of Antibody Buffer

a. Glycerol: It lowers the freezing point below -20°C, so 50% glycerol is usually added to avoid repeated freezing and thawing.

b. Bovine Serum Albumin (BSA): It is added as a stabilizer because protein is less likely to degrade at high concentrations (1 mg/mL or higher). However, if the antibody is to be used forconjugation, BSA should be avoided since it will compete with antibodies for binding to antigens.

c. Sodium Azide: It is used to prevent microbial contamination. However, this substance is toxic to living cells and is therefore not suitable for staining or treating living cells. In addition, sodium azide interferes with most of conjugations that contain an amino group. In this case, thimerosal can be used as a protective agent instead of sodium azide.

6 Selection of Primary Antibodies

a. Type of experiments. The "Application" section of the MCE official website lists types of experiments the very antibody has been tested for, e.g., WB, IHC, ICC, ELISA, FCM, etc.

b. Target antigen species. the MCE website lists which animal/human specimens the very antibody has been tested and validated for.

c. Antibody host species. The MCE official website lists the host species of the very antibody in the "Host" section.

d. Structure of the target protein. ① Protein region: confirm that the antigen (full-length protein, protein fragment, peptide, whole organism or cell) corresponds to or is contained within the protein region to be tested. ② Sample extraction: some antibodies require that the sample should be processed in a specific way. Other antibodies recognize only reduced and denatured proteins whereas a specific group of antibodies recognize only epitopes on proteins in their natural, folded state.

7 Selection of Secondary Antibodies

a. Species of primary antibody. The reacting species of the secondary antibody should be the same as the species of origin of the primary antibody.

b. The class or subclass of the secondary antibody and the primary antibody. Polyclonal antibodies are generally mostly of IgG type, while monoclonal antibodies have different classes and subtypes.

c. Conjugation of the secondary antibody. Horseradish peroxidase (HRP), alkaline phosphatase (AP), fluorescent groups (FITC, APC, PE), and biotin are very common conjugation biomolecules of secondary antibodies. Enzyme-conjugated secondary antibodies are commonly used in Western Blot, IHC, and ELISA, while fluorophore-conjugated secondary antibodies are mostly used in immunofluorescence and flow cytometry.

d. Fragments of secondary antibodies. Common secondary antibody fragments are IgG, Fab fragment, F(ab')2 fragment, etc.

8 Applicable Species of Antibodies

The cross-reactivity and application shown on the MCE official website of certain antibodies are validated. For species that have not been validated, please compare the homology of the antigen sequence of the antibody and the target protein. If the homology is more than 85%, the antibody has a high probability of detecting the target protein, but the successful detection cannot be 100% guaranteed.

9 Dilution Ratio of Antibodies

The recommended dilution ratios are shown on the MCE official website, e.g. 1:1,000. Some of them are written in concentration form, e.g. 1 μg/mL, please calculate the dilution volume ratio based on the antibody stock solution concentration.

10 Selection of the Appropriate Internal Control

Internal control means that an endogenously expressed protein from the same sample is selected as a reference. Please refer to the following principles for the selection of internal control antibodies.

a. Species of the sample. For mammalian tissues or cell samples, β-actin, β-tubulin, GAPDH, Lamin B, Histone H3, etc. are usually selected. Other rare species sources can refer to literature for guidance or select antibodies corresponding to proteins with highly conserved housekeeping gene expression as internal control.

b. Molecular weight of the target protein. The difference between the molecular weight of the target protein and that of the internal control protein should usually be more than 5 kDa (not too large).

c. Target protein expression site. For general protein assay, GAPDH, β-actin or β-tubulin are usually selected; For subcellular organelle proteins, the selection of the corresponding subcellular organelle internal reference can better reflect the accuracy of internal control; For membrane proteins, ATP1A1 is commonly selected as the internal control antibody; For mitochondrial proteins, VDAC1 and COX1 are commonly selected.

d. Special experimental conditions. In some cases, the expression of housekeeping genes may change, and the selection of internal control antibodies should be adjusted accordingly.

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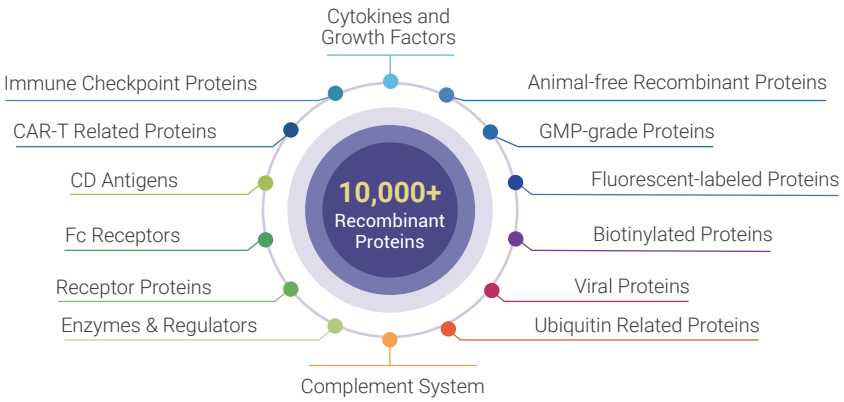

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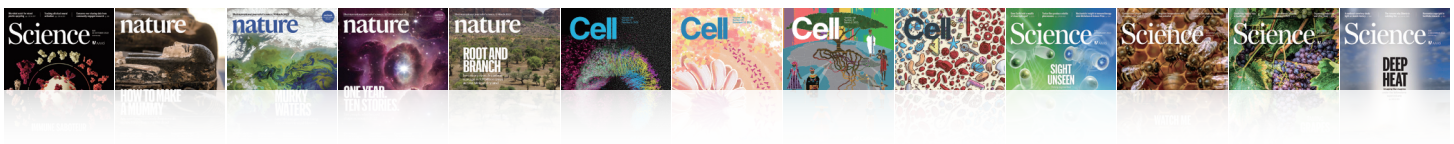

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