

Inhibitory Antibodies User Guide

1 Inhibitory Antibodies Products Classification

- | | |
|------------------------|-----------------------|
| a. Human Antibody | b. Humanized Antibody |
| c. Chimeric Antibody | d. Mouse Antibody |
| e. Bispecific Antibody | f. Fusion Protein |
| g. Isotype Control | h. Nanobody |

2 Inhibitory Antibodies Production Process

The MCE Inhibitory Antibodies are primarily recombinant antibodies, with the exception of some Mouse Antibodies. These monoclonal antibodies are meticulously produced using in vitro synthesized antibody genes. These synthetic antibody genes are modified and recombined by recombinant DNA technology, constructed in plasmids, and transferred to appropriate mammalian cell lines for expression.

3 Inhibitory Antibodies Shipment

MCE Inhibitory Antibodies products are available in blue ice shipments or dry ice shipments. The specific shipping conditions for each product are meticulously detailed on our official website.

4 Inhibitory Antibodies Storage

The product's CoA indicates specific storage conditions and precautions to ensure proper storage. Generally, products stored under recommended conditions can be stably preserved for 2 years.

The product can be reconstituted/diluted with sterile PBS or 0.9% saline. It is recommended that the reconstituted/diluted product be used within one month. During the dissolution, rapid shaking with a vortex oscillator will destroy the spatial structure of antibodies. It is recommended to use a syringe or pipette to mix gently.

When reconstituting/diluting the inhibitory antibody product, it is essential to maintain sterility. This involves using a biosafety cabinet and sterile pipette tips, tubes, syringes and buffer solutions. Avoid storing Inhibitory Antibodies products at -80°C for more than 1 day at 4°C after dilution.

5 Pre-treatment before Opening the Cap

Please ensure to handle the tubes properly before opening the cap for use. During shipment, powder or solution may stick to the walls or caps of the tubes. Therefore, before using, do not open the caps. Instead, centrifuge the tubes at approximately 13,000 rpm for 20-30 seconds to collect the antibodies that may have attached to the caps or walls at the bottom of the tubes. MCE guarantees that the total amount of antibodies in each tube will be as labeled.

6 Common Component Types and Functions in Inhibitory Antibodies

| Component | Function | Typical Representatives |
|------------------------------------|--|---|
| Buffer System | Commonly used buffers maintain a pH value between 4.7 and 7.4. | Acetates, citrates, histidine, proline, succinates, phosphates, Tris (hydroxymethyl) aminomethane |
| Surfactant | <ol style="list-style-type: none"> 1. Locate at the water-air interface to prevent the antibody protein from being adsorbed and then aggregated; 2. Bind with antibodies in the solution to prevent antibody interactions; 3. Change the interaction between antibodies and the inner surface of packaging materials. | Polysorbate 80 (Tween 80), Polysorbate 20 (Tween 20), Poloxamer 188 |
| Excipient | <ol style="list-style-type: none"> 1. Increase antibody surface area, inhibit free energy exchange, and reduce protein aggregation; 2. Acts as a cryoprotectant to stabilise antibody conformation and prevent crystallisation during cryopreservation | Mannitol, sorbitol, sucrose, Trehalose, dextran 40, histidine |
| Osmotic Pressure Regulation | Adjust osmotic pressure | Sodium chloride (NaCl) |
| Antioxidant | <ol style="list-style-type: none"> 1. Reduce metal-catalyzed oxidation reactions; 2. Decrease free radicals and reduce the concentration of oxidants. | Ascorbic acid, methionine, ethylenediamine tetraacetic acid (EDTA) |

The specific components of the inhibitory antibody can be found in the Certificate of Analysis (CoA) information of formulation.

7 Calculation of Inhibitory Antibodies' Concentration

Protein's ultraviolet absorbing property comes from aromatic amino acids such as tryptophan and tyrosine, which contain conjugated double bonds and have the highest light absorption peak near the 280 nm wavelength. Since most proteins/antibodies contain tyrosine and tryptophan residues, measuring the absorbance of proteins/antibodies at a wavelength of 280 nm is the quickest and easiest method for analyzing the protein/antibody content in a solution.

The concentration of MCE Inhibitory Antibody products is calculated A280/extinction coefficient calculation method.

8 What is an Isotype Control Antibody? How to Choose?

Isotype Control is an immunoglobulin of the same origin, subtype, and dose as the test antibody to eliminate background signals due to non-specific binding of the antibody. The Isotype Control also serves as a negative control, primarily to determine that the binding of the primary antibody is specific and not due to non-specific interactions with Fc receptors or other proteins. For example, the antibody type Adalimumab (HY-P9908) is Human IgG1, and we can choose Human IgG1 kappa, Isotype Control (HY-P99001) as its isotype control antibody.

10 Are Inhibitory Antibodies suitable for in vivo Administration in Animals?

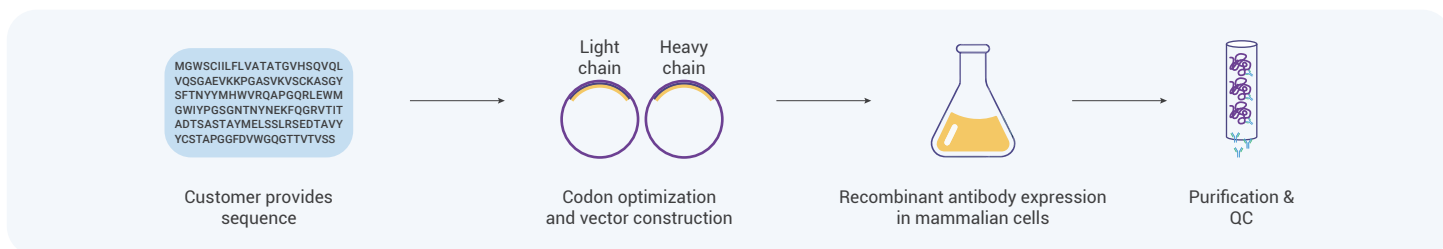
All MCE Inhibitory Antibody products can be used for in vivo animal experiments. All antibodies are prepared in a sterile buffer solution, with a purity $\geq 95\%$ and endotoxin levels < 1 EU/mg.

11 Recombinant Antibody Expression Services

Recombinant antibodies are specific antibodies produced in host cells by genetic engineering techniques.

They are commonly used in areas such as medical research, diagnosis and therapy. Recombinant antibodies have higher specificity and consistency than traditional antibody production methods.

MCE has developed practical methods and tools based on a mammalian cell recombinant expression platform to express recombinant monoclonal antibodies targeting antibody sequences, achieving high yield, high purity, and low endotoxin levels. In addition to expressing full-length antibodies, MCE can also produce various types of recombinant antibodies, including Fab, scFv, VHH, fusion proteins, chimeric antibodies, and bispecific antibodies. MCE's recombinant antibody expression services encompass steps such as codon optimization, vector construction, expression, purification, and QC analysis. You only need to provide the sequence information of the antibody (or VH/VL). We focus on delivering high-quality recombinant antibodies to support your research, ensuring that you can trust the results of your work.



9 What are the Species of Inhibitory Antibodies?

1) **Chimeric antibody:** this antibody combines the variable region of heterologous antibodies with the constant region of human antibodies. For instance, the human-mouse antibody has $>65\%$ human amino acid sequence.

2) **Humanized antibody:** The mouse framework region is replaced by a human framework region, retaining only 3 mouse-derived CDRs. The rest are human structures with $> 90\%$ human amino acid sequence. The antibody is modified to maintain the affinity and specificity of the parental mouse monoclonal antibody, reducing its heterogeneity, which is beneficial for use in humans.

3) **Human antibody:** Full human sequence.

4) **Mouse antibody:** Full mouse sequence.

5) **Other species:** Felinized antibody, Canine/Caninized antibody, Monkey antibody, etc.

Chimeric, human, and humanized antibodies in animal experiments are suitable for humanized mouse/humanized animal models such as nude mice and immunodeficient mice.

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