Isotyping Kit for Mouse Monoclonal Antibody

Description

Code APK001

Size 10 Plates

The Isotyping Kit for Mouse Monoclonal Antibody (APK001) is a powerful research tool designed for qualitative isotype determination of mouse immunoglobulins. This kit enables accurate identification of mouse immunoglobulin isotypes, including IgG1, IgG2a, IgG2b, IgG3, IgM, and kappa from hybridoma cell culture supernatant or purified antibodies by Enzyme Linked Immunosorbent Assay (ELISA). This kit consists of Goat Fc fused nanobody against mouse antibody isotypes, with higher specificity and sensitivity than most available products.

Materials provided

Detection Antibodies	Volume
Anti-Mouse IgG1, AlpHcAbs® Goat antibody(HRP)	50 μL
Anti-Mouse IgG2a, AlpHcAbs® Goat antibody(HRP)	50μL
Anti-Mouse IgG2b, AlpHcAbs® Goat antibody(HRP)	50 μL
Anti-Mouse IgG3, AlpHcAbs® Goat antibody(HRP)	50 μL
Anti-Mouse kappa, AlpHcAbs® Goat antibody(HRP)	50μL
Anti-Mouse IgM, AlpHcAbs® Goat antibody(HRP)	50μL

Note:

• Sufficient reagents are provided to perform over 5000 Clones by ELISA.

• All the Detection Antibodies should be diluted 1:10000 in dilution buffer before use.

• All the Detection Antibodies should be protected from prolonged exposure to light.

Benefits

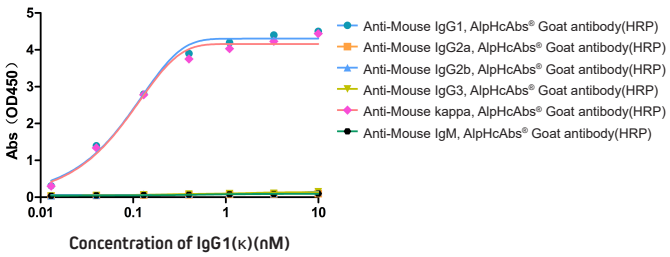
Guaranteed 100% Accuracy
No background
Monoclonal Ab
High lot-to-lot consistency
Increased sensitivity and higher affinity
Animal-free production

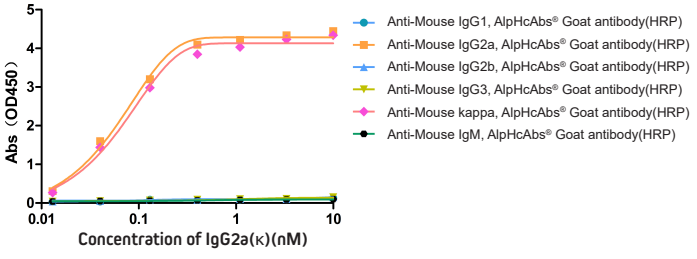
Procedures

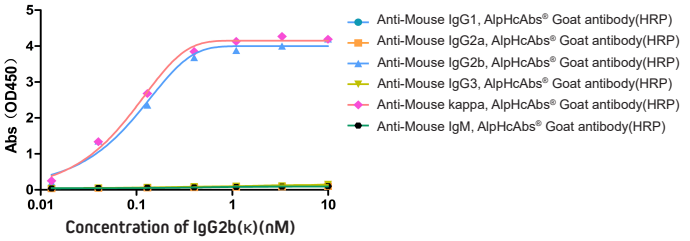
Please read instructions carefully and plan the procedure carefully to achieve the maximum use from this set of isotype specific reagents. The assay should be carried out at room temperature(20–37 ℃).
Notes: Polystyrene multiwell plates from various manufacturers may show differences in absorption properties and considerable lot-to-lot variations; therefore, it is recommended that an approved multiwell plate be used.

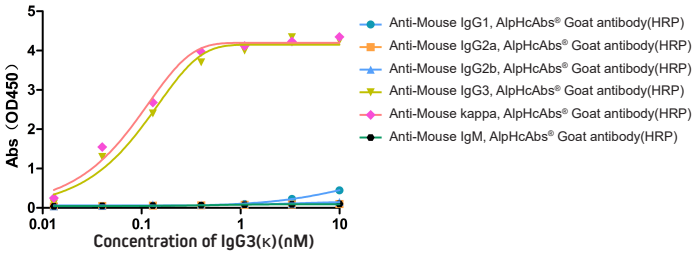
specificity

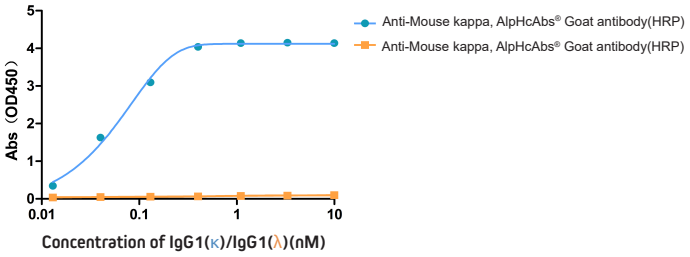
The Isotyping Kit for Mouse Monoclonal Antibody is highly specific, capable of identifying every subtype and isotype of antibodies existing in Hybridoma cell supernatant or purified forms. All the isotype specific antibodies used in the kit are well characterised, and have no cross-reactivity with other isotypes.

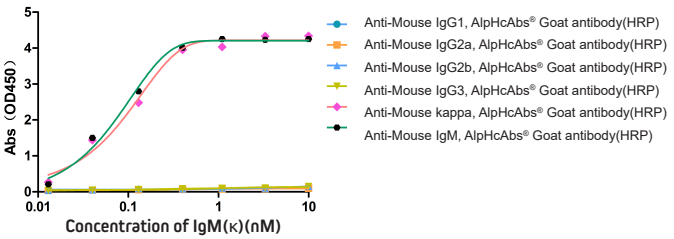












Note:

- Samples were coated on Polystyrene multiwell plates under 37℃ for 2 hours, and detected by Isotyping Kit for Mouse Monoclonal Antibody.
- All the Detection Antibodies were diluted 1:10000 in dilution buffer before use.

Solutions, Reagents and Equipment

PBS: 10mM PBS, pH7.4
Wash buffer: 0.05% Tween20 in PBS, pH 7.2 -7.4
Block buffer: 2% BSA in wash buffer, pH 7.2 -7.4, 0.2 μm filtered
Dilution buffer: 0.1% BSA in wash buffer, pH 7.2 -7.4, 0.2 μm filtered
Substrate solution: To achieve best assay results, fresh substrate solution is recommended
Stop Solution: 1M HCl

Procedure for ELISA

1. Dilute the antigen to 1-2ug/mL in PBS. Immediately coat 96-well microplate with 100 μL per well of the diluted antigen. Incubate the plate overnight at 4℃ or 2 hours at 37℃.
2. Aspirate each well and wash with at least 300 μL wash buffer, repeating the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 μL of blocking buffer to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration / wash as in step 2. The plates are now ready for sample addition.
5. Add 100 μL/well of your samples to the wells (culture supernatant needs no dilution, dilute concentrated or purified samples in dilution buffer to 1-2 μg/mL). Add 100 μL/well of culture medium or dilution buffer into the corresponding wells as Negative Control. Incubate the plate at room temperature for 1 hour.
6. Repeat the aspiration / wash as in step 2.
7. Dilute the Detection Antibodies 1:10000 in dilution buffer. Add 100 μL of the diluted Detection Antibody to each well. Incubate the plate at room temperature for 1 hour.
8. Repeat the aspiration / wash as in step 2.
9. Add 100 μL of substrate solution to each well. Incubate for 10 minutes at room temperature. Avoid placing the plate in direct light.
10. Add 50 μL of stop solution to each well. Gently tap the plate to ensure thorough mixing.
11. Determine the optical density of each well immediately, using a microplate reader set to 450nm.

Results

The antibody isotypes are visibly identified in ELISA applications. The high Optical Density (450nm) suggests the right antibody isotype or subtype. Nevertheless, given the nature of samples being evaluated, in many cases careful attention must be paid when the results are being interpreted.