Mouse Monoclonal Antibody Isotyping Reagents

Catalog Number: SEK003

Storage Temperature: 2-8 °C

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Description

Mouse Monoclonal Antibody Isotyping Reagents (MMAIR) are the research tool intended for qualitative isotype determination of mouse immunoglobulins. This new generation product enables accurate identification of mouse immunoglobulin isotypes, including IgG1, IgG2a, IgG2b, IgG3, and IgM, from hybridoma cell culture supernatant or purified antibodies by capture Enzyme Linked Immunosorbent Assay (ELISA). The tool consists of rabbit monoclonal antibodies against mouse antibody isotypes, and has a higher specificity and sensitivity than most similar products available. In case mixed mouse hybridoma cell cultures, the IRMMA can quantitatively determine each antibody isotype, which is more effective and accurate than antigen based antibody detection methods.

Materials provided

Items	100 tests	1000 tests
Rabbit Anti-Mouse IgG1	30ul	300 ul
Rabbit Anti-Mouse IgG2a	30 ul	80 ul
Rabbit Anti-Mouse IgG2b	30 ul	300 ul
Rabbit Anti-Mouse IgG3	30 ul	300 ul
Rabbit Anti-Mouse IgM	30 ul	80 ul
HRP conjugated Rabbit anti-mouse IgG	30 ul	100 ul

Note: Dilution Ratio differs in detecting different isotypes.

Dilution ratio 1:1000 in PBS is appropriate for IgG1, IgG2b, and IgG3. 1:5000 in PBS is for IgG2a and IgM. HRP conjugated Rabbit anti-mouse IgG should be diluted 1:3000~1:10000 before use.

Specificity

Pronounced superiority of this Mouse Monoclonal Antibody Isotyping Kits is high specific, capable of identifying every subtype and isotype of antibodies existing in Hybridoma cell supernatant or purified forms through reacting with the Fc segments of target antibodies Comparison tests revealed much higher specifity of this Elisa kits than some other noteble companies produced.

Storage / Stability

The kit ships on wet ice and for continuous use, store at 2–8℃.For extended storage, the solutions may be frozen in workingaliquots. Repeated freezing and thawing is not recommended. Storage in “frost-free” freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify thesolution by centrifugation before use.

Declaration

This product is intended for research purposes only. It is not intended to be used for therapeutic or diagnostic purposes in humans or animals.
Any use of this product without the express written authorization of Sino Biological is strictly prohibited.
Recommended work concentration of this product, which is much higher than that used in experiments getting results shown in our web page, guarantee both little background and high experiment stability.

Procedures

The procedures describe the Capture ELISA which is recommended for experiments of purified form and hybridoma cell culture supanatent and Antigen-Mediated Elisa which is recommended for ascites fluid. All ELISA results are qualitative and should be observed visually. Please read instructions carefully and plan the procedure carefully to achieve the maximum use from this set of isotype specific reagents. All pipettes must be accurate. The assay should be carried out at room temperature (20–25 °C).
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.

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, 0.2μm filtered

Sample dilution buffer -0.1% BSA in wash buffer, pH 7.2 -7.4, 0.2μm filtered

Substrate Solution： To achieve best assay results, fresh substrate solution is recommended

Substrate stock solution-10mg/ml TMB (Tetramethylbenzidine) in DMSO**Substrate dilution buffer** -0.05M Na2HPO4 and 0.025M citricacid; adjust pH to 5.5 **Substrate working solution** －For each plate dilute 250μl substrate stock solution in 25ml substrate dilution buffer and then add 80μl 0.75% H2O2, mix it well.

Stop Solution -2 N H2SO4

Procedure for Capture ELISA

1. Dilute the isotype specific antibodies in PBS (0.2 ml of each diluted antibody is needed for each sample to be tested). Dilution ratio 1:1000 in PBS for IgG1, IgG2b, and IgG3. Dilution ratio 1:5000 in PBS for IgG2a and IgM. Immediately coat a 96-well microplate with 100ul per well of the diluted antibody. Incubate the plate (covered) overnight at 4° C or 2 hours at 37° C.
2. Aspirate each well and wash with at least 300μl 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 300μL of blocking buffer to each well. Incubate at room temperature for a minimum of 1hour.
4. Repeat the aspiration / wash as in step 2. The plates are now ready for sample addition.
5. Pipette 0.1 ml of the sample to be tested into each of the wells (use culture supernatant undiluted, dilute concentrated or purified samples diluted in Sample dilution buffer to 1-2 ug / ml). Incubate the plate at room temperature for 1 hour.
6. Repeat the aspiration / wash as in step 2.
7. Dilute the peroxidase labeled Rabbit Anti-Mouse IgG antibody 1:3000~1:10000in Sample dilution buffer. Add 100ul of the enzyme conjugated antibody to each well. Incubate the plate at room temperature for 1 hour.
8. Repeat the aspiration / wash as in step 2.
9. Add 200μL of substrate solution to each well. Incubate for 10 minutes at room temperature **(if substrate solution is not as requested, the incubation time should be optimized)**. Avoid placing the plate in direct light.
10. Add 50μL 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.

References

Engvall E, et al. (1971)Enzyme-linked immunosorbent assay (ELISA). Quantitative assay of immunoglobulin G. Immunochemistry. 8 (9): 871–4.

Leng S, et al. (2008)Elisa and Multiplex Technologies for Cytokine Measurement in Inflammation and Aging Research. J Gerontol a Biol Sci Med Sci. 63 (8): 879–84.

MedLinePlus. (2007) HIV ELISA/western blot. U.S. National Library of Medicine.

Lequin R. (2005)Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA). Clin Chem. 51 (12): 2415–8.

Trouble Shooting

Problem	Possible Sources	Solutions
No color obtained or signal is too weak.	Enzyme labeled second antibody other than recommended.	Use the recommended source for second antibody or determine working dilution for second antibody.
	Concentration of antibody tested too low.	Use higher concentration of sample.
	Substrate solution was not added or prepared incorrectly.	Add substrate solution and continue or Check substrate with peroxidase reagent.
	Inappropriate preservative such as sodium azide is present in the buffer.	Check buffer composition.
	Incorrect storage condition	Check protocol for storage.
Too many signals obtained.	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	More than one hybridoma in the sample.	Reclone the hybridoma.
	Ascites fluid contains host-derived antibodies.	Apply purified sample. Use higher dilution of ascites fluid to dilute out contaminants or switch an antigen-mediated ELISA.
	IgG2b specimen is singled out also by the IgG2a specific antibody giving positive results.	Disregard the IgG2a signal as this background appears occasionally. Consider only the IgG2b signal.
	Enzyme labeled second antibody is other than recommended, resulting in non-specific binding.	Use the recommended source for for the antibody or determine the working dilution for the antibody used.