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Sunlong Medical™ Mouse Ig Isotyping ELISA Kit

Catalog Number: EL0002Mo

Size: 48 Test, 96 Test

Storage: 2-8℃

Validity Period: Two Years

Sensitivity:1.37 ng/mL

Assay range:15.63 ng/mL - 1000 ng/mL

For the **quantitative determination** of mouse Ig isotypes in **hybridoma supernates, ascites and purified antibodies**.

This package insert must be read entirely before using this product. For proper performance, follow the protocol provided with each individual kit.

FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.

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ASSAY PROCEDURE SUMMARY

1. Prepare all reagents and standards as directed.

2. Add 100 µl of *Positive Control* to each well in plate column 1. Add 100 µl *Assay Buffer (1×)* as negative control to each well in column 2.

3. Add 100 µl of sample with appropriate dilution to per well in each plate column.

4. Add 50 µl diluted *Detect Antibody* to each well. Step 2, 3 and 4 should be completed within 15 minutes.

5. Incubate for 2 hours at RT.

6. Aspirate and wash 6 times.

7. Add 100 µl *Substrate Solution* to each well. Incubate for 5 - 30 minutes at RT. Protect from light.

8. Add 100 µl *Stop Solution* to each well.

9. Read at 450 nm within 30 minutes. Correction 570 or 630 nm.

DESCRIPTION

Antibody isotyping is a critical and beneficial aspect of hybridoma development. This kit can identify six immunoglobulin heavy chain isotypes and two light chain isotypes in mice: IgA, IgM, IgG1, IgG2a, IgG2b, IgG3, kappa chain and lambda chain. It can accurately and specifically identify which heavy and light chain of the hybridoma samples is producing and if it is monoclonal or not. This kit is a powerful tool to isolate and characterize each potential clone.

Identification is essential since chemical and biological properties of the various classes are unique. They differ in their solubility and electrophoretic properties, susceptibility to cleavage enzymes, and reactivity with protein A. Determining the class and subclass of a monoclonal antibody is thus useful in planning the best immunoglobulin purification method. For example, mouse IgA and IgM are best purified by size (i.e., gel exclusion) or using immunoaffinity separation columns. Mouse IgG2a and IgG2b are purified with immobilized Protein A at pH 7 - 8, while mouse IgG1 binds best to Protein A at pH 8 - 9. Immunoglobulin that contains kappa light chains can be purified using immobilized Protein L.

PRINCIPLE OF THE ASSAY

This assay employs the qualitative sandwich enzyme immunoassay technique. Monoclonal antibodies specific for mouse Ig isotypes have been pre-coated onto a microplate. Samples, positive control and HRP-linked detect antibody are pipetted into the wells and Ig present is bound by the immobilized antibody and detect antibody following incubation. After washing away any unbound substances, substrate solution is added to the wells and color development is performed. Finally it is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- ⊕ FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- ⊕ Do not use expired kit or reagents.
- ⊕ Do not use reagents from other lots or manufacturers. Do not prepare component by yourself.
- ⊕ Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.
- ⊕ Disposable pipette tips, tubes or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergents before use.
- ⊕ Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Empty wells completely before dispensing fresh wash solution, fill with Washing Buffer as indicated for each wash cycle and do not allow wells to sit uncovered or dry for extended periods.

MATERIALS PROVIDED (96 Test)

Unopened kit should be stored at 2 - 8°C.



- ④ **Ig Isotyping Microplate** (1 plate): 96-well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against mouse Ig isotypes.
- ④ **Ig Isotyping Detect Antibody** (1 vial, 80 µl): HRP-conjugate anti-mouse Ig detect antibody; 100×liquid.
- ④ **Positive Control:** mouse IgG1, IgG2a, IgG2b, IgG3, IgA, IgM, kappa and lambda isotype control mix.
- ④ **Assay Buffer (10×)** (1 bottle, 5 ml): PBS with 0.5 % Tween-20 and 5 % BSA.
- ④ **Substrate** (1 bottle, 15 ml): TMB (tetramethyl-benzidine).
- ④ **Stop Solution** (1 bottle, 15 ml): 0.18 M sulfuric acid.
- ④ **Washing Buffer (20×)** (1 bottle, 50 ml): PBS with 1 % Tween-20.
- ④ **Plate Covers** (5 strips).

STORAGE

Store kit reagents between 2 and 8°C. Immediately after use remaining reagents should be returned to cold storage (2 to 8°C). Expiry of the kit and reagents is stated on labels.

Expiration date of the kit components can only be guaranteed if the components are stored properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

Unopened kit		Store at 2 - 8°C (See expiration date on the label).
Opened/ Reconstituted Reagents	1×Washing Buffer 1×Assay Buffer Stop Solution Substrate TMB Detect Antibody	Up to 1 month at 2 - 8°C.
	Positive Control	Up to 1 month at ≤ -20°C in a manual defrost freezer. Discard after use.
	Microplate Wells	Up to 1 month at 2 - 8°C. Return unused strips to the foil pouch containing the desiccant pack, reseal along entire edge to maintain plate integrity.

Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- ④ **Microplate reader** capable of measuring absorbance at 450 nm, with correction wavelength set at 570 nm or 630 nm.
- ④ **Pipettes and pipette tips.**
- ④ 50 µl to 300 µl adjustable **multichannel micropipette** with disposable tips.
- ④ Multichannel micropipette **reservoir**.
- ④ **Beakers, flasks, cylinders** necessary for preparation of reagents.
- ④ **Deionized or distilled water.**
- ④ **Polypropylene** test tubes for dilution.

PRECAUTION

- ④ All chemicals should be considered as potentially hazardous.
- ④ We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves.
- ④ Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- ④ The Stop Solution provided with this kit is an acid solution. Wear eyes, hand, face, and clothing protection when using this material.
- ④ Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- ④ Do not mix or substitute reagents with those from other lots or other sources.
- ④ Do not use kit reagents beyond expiration date on label.
- ④ Do not expose kit reagents to strong light during storage and incubation.
- ④ Do not eat or smoke in areas where kit reagents or samples are handled.
- ④ Avoid contact of skin or mucous membranes with kit reagents or specimens.
- ④ Rubber or disposable latex gloves should be worn while handling kit reagents or specimens.
- ④ Avoid contact of substrate solution with oxidizing agents and metal.
- ④ Avoid splashing or generation of aerosols.
- ④ In order to avoid microbial contamination or cross- contamination of reagents or specimens which may invalidate the test use disposable pipette tips and/or pipettes.
- ④ Use clean, dedicated reagent trays for dispensing the conjugate and substrate reagent.
- ④ Exposure to acid inactivates the HRP and antibody conjugate.
- ④ Glass-distilled water or deionized water must be used for reagent preparation.
- ④ Substrate solution must be warmed to room temperature prior to use.
- ④ Decontaminate and dispose specimens and all potentially contaminated materials as they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- ④ Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0 % sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

TECHNICAL HINTS

- ④ When mixing or reconstituting protein solutions, always avoid foaming.
- ④ To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- ④ When using an automated plate washer, adding a 30 seconds soak period before washing step and/or rotating the plate between wash steps may improve assay precision.
- ④ To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- ④ Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- ④ Stop Solution should be added to the plate in the same order as the Substrate Solution.
- ④ The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.
- ④ Take care not to scratch the inner surface of the microwells.

SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation at $300 \times g$ for 10 minutes and assay immediately or aliquot and store samples at $\leq -20^{\circ}\text{C}$.

Other biological samples might be suitable for use in the assay. Cell culture supernates, ascites and purified antibodies were tested with this assay.

Note: Samples containing a visible precipitate must be clarified prior to use in the assay. Do not use grossly hemolyzed or lipemic specimens.

Samples should be aliquoted and must be stored frozen at -20°C to avoid loss of bioactive mouse Ig (H+L). If samples are to be run within 24 hours, they may be stored at 2 to 8°C .

Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

REAGENT PREPARATION

Bring all reagents and samples to room temperature before use.

If crystals form in the Buffer Concentrates, warm and gently stir them until completely dissolved.

Washing Buffer (1×)

Pour entire contents (50 ml) of the **Washing Buffer (20×)** into a clean 1000 ml graduated cylinder. Bring to final volume of 1000 ml with pure or deionized water.

Mix gently to avoid foaming.

Transfer to a clean wash bottle and store at 2 to 25°C. Washing Buffer (1×) is stable for 30 days.

Assay Buffer (1×)

Pour the entire contents (5 ml) of the **Assay Buffer (10×)** into a clean 100 ml graduated cylinder.

Bring to final volume of 50 ml with distilled water. Mix gently to avoid foaming.

Store at 2 to 8°C. Assay Buffer (1×) is stable for 30 days.

Detect Antibody

Mix well prior to making dilutions.

Make a **1: 100** dilution of the concentrated **Detect Antibody** solution with Assay Buffer (1×) in a clean plastic tube as needed.

The diluted Detect Antibody should be used within 30 minutes after dilution.

Sample Dilution

If your samples have high Ig content, Assay Buffer (1×) is used for dilution of cell culture medium, ascites and purified antibodies

Mouse Ig Positive Control

Reconstitute **Mouse Ig Positive Control** by addition of distilled water. Reconstitution volume is stated on the label of the standard vial. Swirl or mix gently to insure complete and homogeneous solubilization.

Allow the standard to reconstitute for 10 - 30 minutes. Mix well prior to making dilutions.

ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use.

1. Prepare all reagents including microplate, samples, standards and working solution as described in the previous sections.
2. Remove excess microplate strips and return them to the foil pouch containing the desiccant pack, and reseal for further use.
3. Add 300 μl *Washing Buffer (1 \times)* per well, and allow it for about 30 seconds before aspiration. Soaking is highly recommended to obtain a good test performance. Empty wells and tap microwell strips on absorbent pad or paper towel to remove excess *Washing Buffer (1 \times)*. Use the microwell strips immediately after washing. **Do not allow wells to dry.**
4. Add 100 μl of *Positive Control* to each well in plate column 1. Add 100 μl of *Assay Buffer (1 \times)* as negative control to each well in plate column 2.
5. Add 100 μl of sample with appropriate dilution to per well in each plate column.
6. Add 50 μl of diluted *Detect Antibody* to each well. Ensure reagent addition in step 4, 5 and 6 is uninterrupted and completed within 15 minutes.
7. Cover with an adhesive strip. Incubate at room temperature (18 to 25°C) for 2 hours on a microplate shaker set at 300 rpm.
8. Aspirate each well and wash, repeating the process five times for a total six washes. Wash by filling each well with 300 μl *Washing Buffer (1 \times)*. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
9. Add 100 μl of *Substrate Solution* to each well. Incubate for 5 - 30 minutes at room temperature. Protect from light.
10. Add 100 μl of *Stop Solution* to each well. The color will turn yellow. If the color in the well is green or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
11. Measure the optical density value within 30 minutes by microplate reader set to 450 nm. If wavelength correction is available, set to 570 nm or 630 nm. If wavelength correction is not available, subtract readings at 570 nm or 630 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Reading directly at 450 nm without correction may generate higher concentration than true value.

PLATE LAYOUT

	1	2	3	4	5	6	7	8	9	10	11	12
A	P	N	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10 IgA
B	P	N	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10 IgG1
C	P	N	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10 IgG2a
D	P	N	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10 IgG2b
E	P	N	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10 IgG3
F	P	N	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10 IgM
G	P	N	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10 Kappa
H	P	N	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10 Lambda

P: Positive control N: Negative control S1-S10: Sample 1 - Sample