

## Technical Data Sheet

## Mouse Immunoglobulin Isotyping ELISA Kit

## Product Information

**Material Number:** 550487  
**Size:** 80 Tests

## Description

The Mouse Immunoglobulin Isotyping ELISA Kit enables rapid, efficient identification of mouse immunoglobulin isotypes. This kit employs a direct horseradish peroxidase-labeled system and the assay format eliminates the need for coating the plate with antigen. These features lead to a significant reduction in assay time without sacrificing sensitivity. Each kit supplies: 8 mouse immunoglobulin isotype-specific rat monoclonal antibodies, a horseradish peroxidase (HRP)-conjugated rat anti-mouse Ig antibody, substrate/stop solutions, coating/blocking buffers and a positive reference antigen mixture. The positive reference antigen mixture is a mixture of purified monoclonal mouse immunoglobulins of nine Ig heavy-and light-chain isotype combinations (IgG1κ, IgG1λ, IgG2ακ, IgG2αλ, IgG2βκ, IgG3κ, IgMκ, IgAκ, and IgAλ).

## Materials provided:

| ITEM                                | AMOUNT  | Working Dilution |
|-------------------------------------|---------|------------------|
| Rat anti-mouse IgG1 purified mAb*   | 1.0 ml  | 1:5              |
| Rat anti-mouse IgG2a purified mAb*  | 1.0 ml  | 1:5              |
| Rat anti-mouse IgG2b purified mAb*  | 1.0 ml  | 1:5              |
| Rat anti-mouse IgG3 purified mAb*   | 1.0 ml  | 1:5              |
| Rat anti-mouse IgM purified mAb*    | 1.0 ml  | 1:5              |
| Rat anti-mouse IgA purified mAb*    | 1.0 ml  | 1:5              |
| Rat anti-mouse Ig κ purified mAb*   | 1.0 ml  | 1:5              |
| Rat anti-mouse Ig λ purified mAb*   | 1.0 ml  | 1:5              |
| HRP-labeled rat anti-mouse Ig Ab†   | 1.0 ml  | 1:100            |
| Substrate Reagent A                 | 40.0 ml | —                |
| Substrate Reagent B                 | 40.0 ml | —                |
| Stop Solution                       | 40.0 ml | —                |
| Positive Reference Antigen Mixture* | 0.25 ml | 1:50             |
| 10X PBS Buffer                      | 40.0 ml | 1:10             |
| 10% BSA** Solution†                 | 30.0 ml | 1:10             |

\* Aqueous buffered solution containing  $\leq 0.09\%$  sodium azide.

\*\* Source of all serum proteins is from USDA inspected abattoirs located in the United States.

† Aqueous buffered solution containing ProClin.

## Preparation and Storage

Store undiluted at 4°C and protected from prolonged exposure to light. Do not freeze.

The monoclonal antibody was purified from tissue culture supernatant or ascites by affinity chromatography.

The antibody was conjugated with HRP under optimum conditions, and unconjugated antibody and free HRP were removed.

## Application Notes

## Application

|       |                  |
|-------|------------------|
| ELISA | Routinely Tested |
|-------|------------------|

## Recommended Assay Procedure:

## Materials Required but not Provided:

96-well ELISA-grade polystyrene or PVC microtiter plates; modular strips are also acceptable

Precision pipettes capable of delivering between 50 and 200 µl

0.05% Tween-20 in PBS as washing solution

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### Reagent Preparation:

1. Bring all reagents to room temperature (18 - 25°C) before use.
2. Coating Buffer (1X PBS): Dilute required quantity of 10X PBS with deionized or distilled water, mix (50 ml for each plate).
3. Blocking Buffer: Dilute required quantity of 10% BSA 1:10 with 1X PBS (35 ml for each plate).
4. Dilute positive reference antigen mixture 1:50 with Blocking Buffer (1 ml for each plate).
5. Dilute HRP-labeled rat anti-mouse Ig Ab 1:100 with Blocking Buffer (10 ml for each plate).
6. Substrate Solution: Within 15 minutes prior to use, mix equal volumes of Substrate Reagent A and Substrate Reagent B (5 ml of each solution for each plate) in a clean glass tube or flask. Make only the amount required for each test. Discard any remaining working solution after use.

### Antibody Coating:

Note: For optimal results, the required amounts of the purified coating antibodies should be diluted immediately before use, and the diluted antibodies should not be stored for a long period of time. Diluted aliquots should not be frozen.

7. Dilute an appropriate amount of each isotype-specific rat anti-mouse purified monoclonal antibody in Coating Buffer and deliver 50 µl of each reagent to applicable rows (see *Figure 1* for suggested layout).
8. Tap plate gently to ensure even distribution of antibody solution on the bottom of wells.
9. Incubate, covered, at 37°C for 1 hour or at 4°C overnight.
10. Use washing solution (0.05% Tween-20 in PBS) to wash out plate contents using a plate washer or similar device and taking care not to cross-contaminate wells with different capture antibodies. Then shake out remaining contents, and blot excess on a clean paper towel. Repeat the wash 3X.
11. Add 200 µl of Blocking Buffer (see Reagent Preparation, Step 3) to each well, and incubate at room temperature for 30 minutes.
12. To prepare for Step 13, wash 3X, shake out Blocking Buffer, and blot dry.

### Sample Incubation:

13. Pipette 100 µl of each sample (e.g hybridoma culture supernatant) to be tested to the appropriate plate columns and incubate for 1 hour at room temperature. Positive controls (see Reagent Preparation, Step 4) should be included as desired; negative controls generally consist of parent myeloma culture supernatant (see *Figure 1* for suggested layout).
14. To prepare for Step 15, wash 3X, shake out remaining contents, and blot dry.

### Enzyme Conjugate Incubation:

15. Pipette 100 µl of HRP-labeled rat anti-mouse Ig mAb solution (see Reagent Preparation, Step 5) to each well, and incubate at room temperature for 1 hour.
16. To prepare for Step 17, wash 6X, soaking the wells for 30 seconds to 1 minute on each wash. Thorough washing at this step is very important.

### Color Development:

17. Add 100 µl of prepared Substrate Solution (see Reagent Preparation, Step 6) to each well and incubate plate for 3 - 10 minutes at room temperature. Positive reaction wells will develop a greenish-blue color. Negative wells will be colorless.
18. Pipette 50µl of Stop Solution to each well. Positive wells will become yellow.

### Plate Result Reading:

19. Read visually or spectrophotometrically at 450 nm. If wavelength correction is available, subtract A (570 nm) from A (450 nm). *Figure 2* is an example of the visual readout for immunoglobulins of various isotypes.

**Danger:** Substrate Reagent B (component 51-04141E) contains 33.05% methanol (w/w).

#### Hazard statements

Flammable liquid and vapour.

Toxic if swallowed, in contact with skin or if inhaled.

Causes damage to the central nervous system. Route of exposure: Oral.

#### Precautionary statements

Keep away from heat/sparks/open flames/hot surfaces. - No smoking.

Wear protective gloves / eye protection.

Wear protective clothing.

Do not breathe mist/vapours/spray.

IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.

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**Danger:** Stop Solution (component 51-04161E) contains 15.23% phosphoric acid (w/w).

**Hazard statements**

Causes severe skin burns and eye damage.

**Precautionary statements**

Wear protective gloves / eye protection.

Wear protective clothing.

IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower.

IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.

Continue rinsing.

IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.

Dispose of contents/container in accordance with local/regional/national/international regulations.

**Warning:** HRP Rat Anti-Mouse Ig (100x) (component 51-04317E) contains 0.004% (w/w) and 10% BSA Solution (component 51-04181E) contains 0.003% (w/w) of a CMIT/MIT mixture (3:1), which is a mixture of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC No 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC No 220-239-6] (3:1).

**Hazard statements**

May cause an allergic skin reaction.

**Precautionary statements**

Wear protective gloves / eye protection.

Wear protective clothing.

Avoid breathing mist/vapours/spray.

If skin irritation or rash occurs: Get medical advice/attention.

IF ON SKIN: Wash with plenty of water.

Dispose of contents/container in accordance with local/regional/national/international regulations.

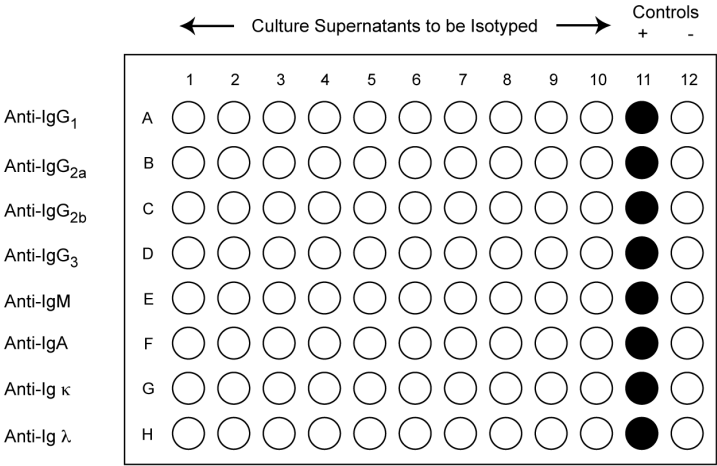


Figure 1. Suggested capture antibody layout (i.e antibody coating).

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|                        |   |                |                |                 |                 |                 |                 |                |                |   |    |    |    |
|------------------------|---|----------------|----------------|-----------------|-----------------|-----------------|-----------------|----------------|----------------|---|----|----|----|
|                        |   | G <sub>1</sub> | G <sub>1</sub> | G <sub>2a</sub> | G <sub>2a</sub> | G <sub>2b</sub> | G <sub>2b</sub> | G <sub>3</sub> | G <sub>3</sub> | M | M  | A  | A  |
|                        |   | κ              | λ              | κ               | λ               | κ               | λ               | κ              | λ              | κ | λ  | κ  | λ  |
|                        |   | 1              | 2              | 3               | 4               | 5               | 6               | 7              | 8              | 9 | 10 | 11 | 12 |
| Anti-IgG <sub>1</sub>  | A | ●              | ●              | ○               | ○               | ○               | ○               | ○              | ○              | ○ | ○  | ○  | ○  |
| Anti-IgG <sub>2a</sub> | B | ○              | ○              | ●               | ●               | ○               | ○               | ○              | ○              | ○ | ○  | ○  | ○  |
| Anti-IgG <sub>2b</sub> | C | ○              | ○              | ○               | ○               | ●               | ●               | ○              | ○              | ○ | ○  | ○  | ○  |
| Anti-IgG <sub>3</sub>  | D | ○              | ○              | ○               | ○               | ○               | ○               | ●              | ●              | ○ | ○  | ○  | ○  |
| Anti-IgM               | E | ○              | ○              | ○               | ○               | ○               | ○               | ○              | ○              | ● | ●  | ○  | ○  |
| Anti-IgA               | F | ○              | ○              | ○               | ○               | ○               | ○               | ○              | ○              | ○ | ○  | ●  | ●  |
| Anti-Ig κ              | G | ●              | ○              | ●               | ○               | ●               | ○               | ●              | ○              | ● | ○  | ●  | ○  |
| Anti-Ig λ              | H | ○              | ●              | ○               | ●               | ○               | ●               | ○              | ●              | ○ | ●  | ○  | ●  |

Figure 2. Expected/Predicted results for immunoglobulin of the indicated isotypes.

## Product Notices

1. ProClin is a trademark of Rohm and Haas Company.
2. Source of all serum proteins is from USDA inspected abattoirs located in the United States.
3. Caution: Sodium azide yields highly toxic hydrazoic acid under acidic conditions. Dilute azide compounds in running water before discarding to avoid accumulation of potentially explosive deposits in plumbing.
4. Please refer to [www.bdbiosciences.com/pharming/protocols](http://www.bdbiosciences.com/pharming/protocols) for technical protocols.

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