Technical Data Sheet

Rat Immunoglobulin Isotyping ELISA Kit

Product Information

Material Number: **557081**
Size: 80 Tests

Description

The Rat Immunoglobulin Isotyping ELISA Kit enables rapid, efficient identification of rat 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 antibody was generated from rat immunoglobulin (Ig) antigen and has been extensively tested for reactivity toward rat Ig isotypes. Each monoclonal antibody is specific for its stated isotype. However, the anti-rat IgG2a antibody pair has been shown to cross-react with rat IgG1 isotype antibodies in some instances. The positive control is a mixture of purified monoclonal rat immunoglobulins of nine Ig heavy- and light-chain isotype combinations (IgG1κ, IgG1λ, IgG2aκ, IgG2aλ, IgG2bκ, IgG2cκ, IgMκ, IgMλ, and IgAκ).

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.

ITEM	AMOUNT	Working Dilution
Mouse anti-rat IgG1 purified mAb*	0.25 ml	1:50
Mouse anti-rat IgG2a purified mAb*	0.25 ml	1:50
Mouse anti-rat IgG2b purified mAb*	0.25 ml	1:50
Mouse anti-rat IgG2c purified mAb*	0.25 ml	1:50
Mouse anti-rat IgM purified mAb*	0.25 ml	1:50
Mouse anti-rat IgA purified mAb*	0.25 ml	1:50
Mouse anti-rat Ig κ purified mAb*	0.25 ml	1:50
Mouse anti-rat Ig λ purified mAb*	0.25 ml	1:50
HRP-labeled mouse anti-rat 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

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.

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).

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5. Dilute HRP-labeled mouse anti-rat Ig Ab 1:100 with Blocking Buffer (12 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 never be stored for a long period. Diluted aliquots should not be frozen.

7. Dilute an appropriate amount of each isotype-specific mouse anti-rat monoclonal antibody in Coating Buffer and deliver 100 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 crosscontaminate 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 hybridoma culture supernatant to be tested to 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 mouse anti-rat 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 50 µl 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.

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 Mouse Anti-Rat Ig (100x) (component 51-04127E) 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.

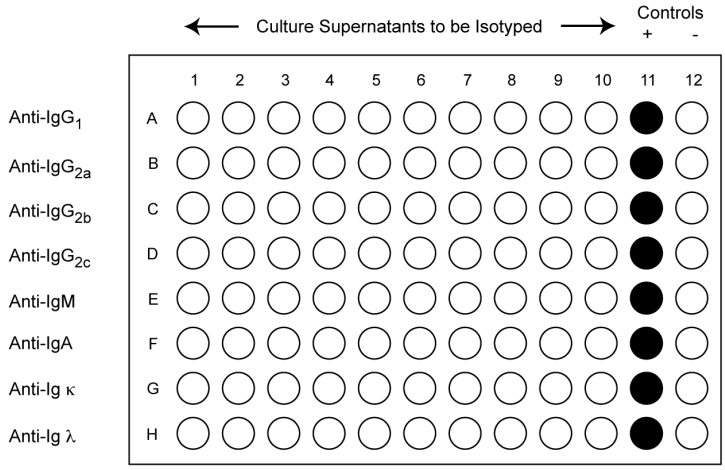

Figure 1. Suggested Capture Layout for mAb-based Ig Isotyping Kit

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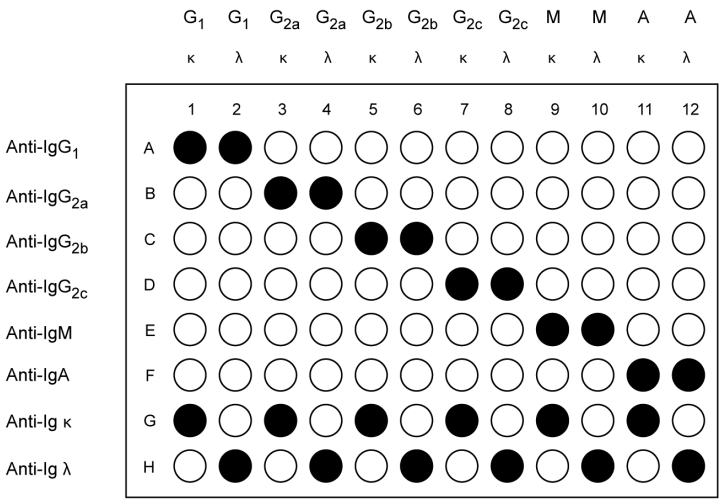

Figure 2. Expected Result with Immunoglobulins of the Indicated Isotypes.

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/pharmingen/protocols for technical protocols.

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