Version 1a Last updated 3 June 2021

ab273149 Mouse IgG Isotyping Kit

View ab273149
Mouse IgG Isotyping Kit datasheet:
www.abcam.com/ab273149
(use www.abcam.cn/ab273149 for China, or www.abcam.co.jp/ab273149 for Japan)

For characterizing mouse monoclonal isotypes in cell culture supernatants or purified antibody preparations.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

The Mouse Immunoglobulin Isotyping Kit (ab273149) provides a rapid and easy method (one step ELISA) to characterize mouse monoclonal antibody isotypes in cell culture supernatants or purified antibodies preparations. The kit includes ready-to-use reagents necessary to analyze 12 samples in less than 30 minutes.

Anti-mouse antibodies specific to each of the common light and heavy chains are pre-coated in the wells. Samples are pipetted into microwells and Ig present in the sample are bound by the capture antibody. Then, an HRP (horseradish peroxidase) conjugated anti-mouse IgG (H+L) antibody is pipetted and incubated simultaneously with samples. After washing microwells in order to remove any non-specific binding, the ready to use substrate solution (TMB) is added to microwells and color develops if the specific immunoglobulin is present in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

2. Protocol Summary

Prepare all reagents and samples as instructed.

Add 20 µL of sample to each well of the strip.

Immediately add 100 µL of peroxidase conjugated anti-mouse IgG to each well. Incubate for 15 mins.

Remove solution and wash three times in wash solution.

Add 100 µL TMB substrate to each well.

After 10 minutes add 100 µL Stop solution.

Results can be directly seen or read at 450nm.

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 4°C immediately upon receipt. Kit has a storage time of 6 months from receipt.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	1 x12 tests	5 X 12 tests	Storage Condition
Pre-coated microwells strips	12 strips of 8 microwells	60 strips of 8 microwells	+4°C
Sample Diluent	30 mL	150 mL	+4°C
Detection antibody	12 mL	60 mL	+4°C
TMB Substrate	12 mL	60 mL	+4°C
Stop Solution	12 mL	60 mL	+4°C

Pre-coated 8 microwell strips have the following well configuration:
Well A: Anti-IgG1
Well B: Anti-IgG2a
Well C: Anti IgG2b
Well D: Anti-IgG3
Well E: Anti-IgM
Well F: Anti-Kappa
Well G: Anti-Lambda chain
Well H: Anti-IgG (H+L)

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- ELISA plate washer
- Wash solution (H_20, 0.05% Tween 20 **or** PBS, 0.05% Tween 20)
- Standard microplate reader - capable of reading absorbance at 405 nm

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- Pre-rinse the pipette tip with the reagent, use fresh pipette tips for each sample and reagent.
- Pipette samples to the bottom of the wells.
- Add the reagents to the side of the tube to avoid contamination.
- Some Solutions supplied in this kit are caustic; care should be taken with their use.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (25°C or 37°C) 30 mins prior to use.
- All reagents are supplied ready to use.

10. Sample Preparation

- Dilute the samples in dilution buffer.
- Recommended dilution for cell culture supernatant is 1/20.
- Recommended concentration for purified Ig is 1 µg/mL.

11. Assay Procedure

– Equilibrate all prepared reagents to desired assay temperature prior to use.

11.1 Transfer 20 µL of diluted samples in each well of the strip

11.2 Immediately add 100 µL of Detection antibody to each well. Mix gently until obtaining a homogeneous purple color.

11.3 Incubate at room temperature for 15 minutes.

11.4 After incubation, remove the solution and wash the wells three times with 300 µL of wash solution.

11.5 Add 100 µL of TMB substrate to each well. Tap plate briefly to mix. Incubate for 10 minutes at room temperature.

11.6 After incubation add 100 µL of stop solution to each well.

11.7 Results can be seen directly or read the absorbance with a microplate reader at 450 nm.

12. Typical Data

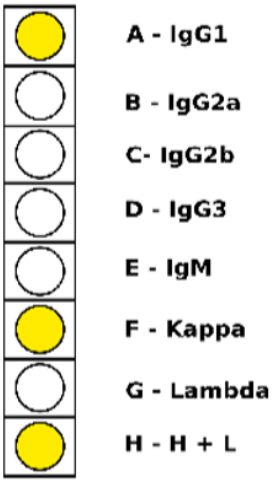

Figure 1. Characterization of a IgG1 antibody: yellow colour is observed in well A (corresponding to IgG1 Heavy chain), well F (corresponding to Kappa Light chain) and well H (for the positive control H+L). The well H has to be positive in order to validate the method

13. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)