MOUSE IMMUNOGLOBULIN ISOTYPING KIT

Catalog number RDB-3255-1 / RDB-3255-5

Data sheet version : 07/04/2023

FastELISA®

APPLICATION

The mouse immunoglobulin isotyping kit 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 up to **12 or 60 samples** (for RDB-3255-1 or RDB3255-5 respectively) in **30 min**.

Buffer solutions are color coded in order to simplify pipetting steps.

PRINCIPLE OF THE ASSAY

The anti-mouse Ig capture antibody pre-coated onto microwells are bound to the mouse IgG present in the sample. A 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 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.

SPECIFICITY AND SENSIVITY

The method enables the detection of Light chains (Kappa and Lambda) and Heavy chains (Gamma 1, Gamma 2a, Gamma 2b, Gamma 3 and Mu) of mouse immunoglobulins.

STORAGE

Storage at 2-8°C. After opening, reagents must be handled with care to avoid contamination and should be used within 2 months. Do not freeze.

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FOR RESEARCH ONLY

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KIT CONTENTS

Code	Component	Quantity 1x96 wells RDB-3255-1	Quantity 5x96 wells RDB-3255-5
RDB3255-P	Pre-coated 8 microplates strips with the following isotypes antibodies: - Wells A: anti-IgG1 - Wells B: anti-IgG2a - Wells B: anti-IgG2b - Wells B: anti-IgG3 - Wells B: anti-IgM - Wells B: anti-Kappa chain - Wells H: anti-IgG (H+L)	12 strips of 8 wells	60 strips of 8 wells
AS31	Sample diluent (**Blue solution**)	30 ml	150 ml
RDB3255-C	Detection antibody (**Red solution**)	12 ml	60 ml
AS29	TMB	12 ml	60 ml
AS40	Stop solution	12 ml	60 ml

ADDITIONNEL MATERIEL REQUIRED

Pipettes (20-200 µl).
ELISA plate washer (recommended).
Microplate reader for absorbance measurements at 450 nm and at 620 nm.
Wash solution: H2O + 0.05% Tween 20 or PBS 1X 0.05% Tween 20.

SAMPLE PREPARATION AND STORAGE

Dilute the samples in the dilution buffer RDB3255-D (blue solution).
Cell culture supernatant: 1:20.
Recommended concentration for purified Ig: 1µg/ml.

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PROCEDURE

All steps must be performed at room temperature (RT). Bring all reagents at RT for 30 min before use.

STEP 1	Add 20µl of diluted samples (**blue solution**) in each well of the strip.
STEP 2	Add without delay in the same order 100 µl of peroxidase conjugated anti-mouse IgG (**red solution**). Mix gently until obtaining a homogeneous **color purple**. Incubate the plate for 15 min at RT.
STEP 3	After incubation, remove the solution and wash the plate three times each with **300 µl** of the wash solution.
STEP 4	Pipette 100 µl of TMB substrate into each well. Incubate the plate for **10 min** at RT.
STEP 5	Stop the reaction by pipetting 100 µl of STOP solution in the same order as for TMB distribution.
STEP 6	Read the absorbance at **450 nm** and 620 nm with a microplate reader.



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INTERPRETATION AND VALIDATION OF RESULTS

Exemple for a caracterisation of a IgG1 Kappa antibody: yellow color 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.

