MOUSE IMMUNOGLOBULIN ISOTYPING ELISA KIT

FastELISA®

Catalog number RDB 3255 - 12 tests

APPLICATION

The RD-Biotech 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 **12 samples in less than 30 minutes**.
Buffer solutions are color coded in order to simplify pipetting steps.

PRINCIPLE OF THE ASSAY

The method employs the quantitative sandwich enzyme immunoassay technique. 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, 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 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.

SPECIFICITY

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

All kit components are stable for 12 months when stored at 2-8°C. Do not freeze.
After opening, reagents must be stored at 2-8°C, handled with care to avoid contamination and should be used within 2 months.

KIT CONTENTS

Code	Component	Quantity
RDB3255-P	Pre-coated 8 microwells strips with the following isotypes antibodies: - Wells A: anti-IgG1 - Wells B: anti-IgG2a - Wells C: anti-IgG2b - Wells D: anti-IgG3 - Wells E: anti-IgM - Wells F: anti-Kappa - Wells G: anti-Lambda chain - Wells H: anti-IgG (H+L)	12 strips of 8 microwells
RDB3255-D	Sample Diluent (PBS pH7.4, 1% BSA, 0.1% Tween 20) (Blue solution)	30 ml
RDB3255-C	Detection antibody: Peroxidase conjugated anti-mouse IgG (H+L) (Red solution)	12 ml
RDB3255-T	TMB Substrate	12 ml
RDB3255-St	Stop solution (2M HCl)	12 ml

All the kit components are ready-to-use

ADDITIONAL MATERIAL REQUIRED

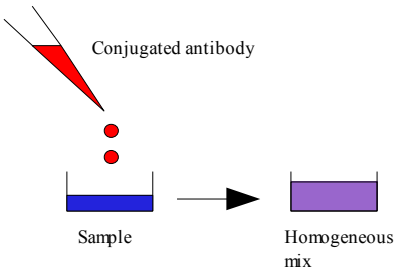
- Pipettes and tips (20-200 µl).
- ELISA plate washer (recommended)
- Microplate reader for absorbance measurements at **450 nm.**
- Wash solution: H2O, 0.05% Tween 20. Other wash solutions may be used but they have to be tested with the method.

SAMPLE PREPARATION

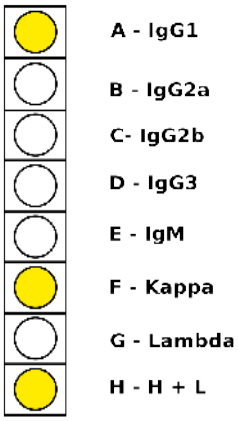
Dilute the samples in the dilution buffer RDB3255-2 (blue solution).
Cell culture supernatant: 1:20
Recommended concentration for purified Ig: 1µg/ml

Assay procedure

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

STEP 1	Add 20 µl of diluted samples (Blue) in each well of the strip.
STEP 2	 Add without delay 100 µl of peroxidase conjugated anti-mouse Ig (Red solution) to each well. Mix gently until obtaining an homogeneous purple colour. Incubate the plate for **15 min** at RT.
STEP 3	After incubation, remove the solution and wash the microwells three times with **300 µl** of the wash solution.
STEP 4	Add 100 µl of TMB substrate in each well. Incubate for **10 min** at room temperature.
STEP 5	Stop the reaction with 100 µl of STOP solution.
STEP 6	Results can be directly seen. The absorbance can also be read with a microplate reader at **450 nm**. The plate can be sealed with adhesive tape and be photographed for permanent record.

TYPICAL RESULT



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.

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