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Mouse Immunoglobulin Easy Isotyping ELISA Kit

Catalog No: IS001A **Quantity:** 12 tests

Catalog No: IS001B **Quantity**: 5 x 12 tests

Application:
The Mouse Immunoglobulin Easy Isotyping ELISA Kit provides a rapid and easy method (one antibody incubation step) 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 is 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 immunoglobulin.

Kit Contents (for 1 x 12 tests):

Item	Description	Quantity
IS001-P	Pre-coated 8 microwell strips with the following isotype 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
IS001-D	Sample Diluent: (PBS pH 7.4, 1% BSA, 0.1% Tween 20) Blue solution	30 ml
IS001-C	Detection antibody: Peroxidase conjugated anti-mouse IgG (H+L) Red solution	12 ml
IS001-T	TMB Substrate	12 ml
IS001-St	Stop solution (2M HCl)	12 ml

All the kit components are ready-to-use.

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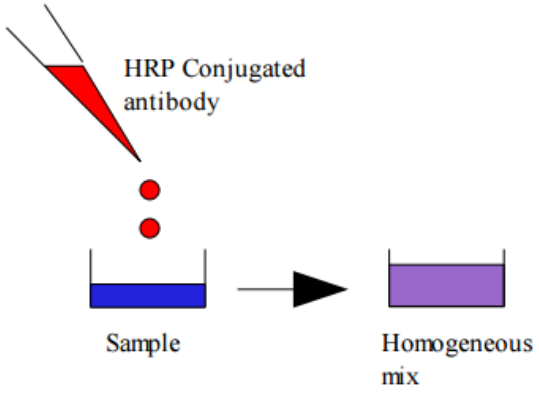
Storage:
All kit components are stable for 12 months when stored at 2-8°C. **Do not freeze.** After opening, reagents must be stored at 2-8°C, handled with care to avoid contamination and should be used within 2 months.

Additional Materials Required:

- Pipettes and tips (20-200 µl)
- ELISA plate washer (recommended)
- Microplate reader for absorbance measurements at 450 nm
- Wash solution: H_2O, 0.05% Tween 20
  Note: Other wash solutions may be used but they have to be tested with the method.

Sample Preparation:
Dilute the samples in the dilution buffer IS001-D (Blue solution).
Cell culture supernatant: 1:20
Recommended concentration for purified Ig: 1 µg/ml

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

Step 1	Add 20 µl of diluted samples (Blue) in each well of the strip.
Step 2	Immediately add 100 µl of peroxidase conjugated anti-mouse Ig (Red solution) to each well. Mix gently to obtain a homogeneous purple color. Incubate the plate for 15 min at room temperature.
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.

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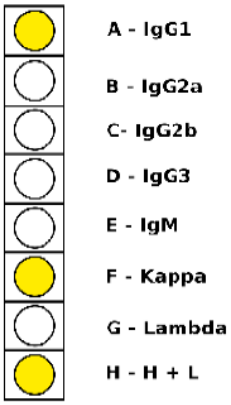
Typical Result:



Characterization of an IgG1 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.

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

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