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Mouse IgG Quantification ELISA Kit

03/17

(Catalog # K4161-100; 100 assays; Store at -20°C)

I. Introduction:

The BioVision's Mouse IgG ELISA Kit is engineered for precise quantification of total mouse IgG in serum, ascites, and hybridoma cell culture supernatant samples. A polyclonal antibody specific for mouse IgG has been pre-coated onto the 96-well microtiter plate to capture mouse IgG in standards and biological samples. TMB substrate is used to detect HRP conjugated to detection antibody specific for mouse IgG. TMB is converted by HRP to a blue color product that changes into yellow color after adding stop solution. The intensity of the color is proportional to the amount of Mouse IgG in samples and can be measured spectrophotometrically at 450 nm.

II. Application:

Quantitative and direct determination of concentration of total mouse IgG in biological fluids without its purification

III. Specificity:

Mouse IgG (including IgG1, IgG2a/IgG2c, IgG2b, and IgG3)
Detection Range: 1 – 100 ng/ml

IV. Sample Type:

Mouse serum, ascites, hybridoma cell culture supernatant and other biological fluids

V. Kit Contents:

Components	K4161-100	Cap Color	Part No.
Pre-coated 96 well strip plate	8X12 strips	--	K4161-100-1
Mouse IgG Standard (100 ng/ml)	500 µl	Yellow	K4161-100-2
Detection Antibody-HRP	50 µl	Orange	K4161-100-3
Diluent Buffer	35 ml	WM	K4161-100-4
TMB Substrate (Avoid light)	10 ml	Amber	K4161-100-5
Stop Solution	11 ml	NM	K4161-100-6
Plate Sealers	2	--	K4161-100-7

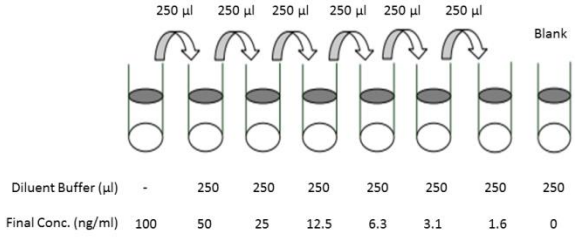
VI. User Supplied Reagents and Equipment:

- Wash Buffer: PBS (pH 7.4) with 0.05% Tween-20 (v/v) (PBST)
- Microplate reader capable of measuring absorbance at 450 nm
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended
- Eppendorf tubes and 15 ml conical tube
- Absorbent paper

VII. Storage Conditions and Reagent Preparation:

Upon arrival, store the kit at -20°C. Stable for 1 month after first use if stored properly. Briefly centrifuge vials prior to opening. Avoid repeated freeze-thaw cycles. Read the entire protocol before performing the assay. Bring all buffers and samples to room temperature (18-25°C) before starting.

- **Mouse IgG standards**: To prepare 0.5 ml of 50 ng/ml standard, gently mix 250 µl of Standard stock solution in 250 µl of Diluent Buffer. Perform 2-fold serial dilutions of the standards to prepare the standard curve within the range of this assay. Suggested standard points are: 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0 ng/ml. Use 250 µl Diluent Buffer as blank. The mouse IgG standard solutions are best used within 60 minutes on ice.



1. **Detection Antibody-HRP**: Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells with additional 0.1 - 0.2 ml of the total volume. Dilute the **Detection Antibody-HRP** with Diluent buffer at 1:200 and mix thoroughly. Prepare reagents within 30 minutes before the experiment.

- **Sample Preparation**: The detection range for this ELISA kit is 1 - 100 ng/ml. For ideal assay conditions, researchers must determine appropriate dilution factor of all samples to ensure that data fits into the linear range. Typically, mouse ascites and serum samples contain 5 - 10 mg/ml of IgG (recommended dilution factor: 1:100,000 – 1000,000), and hybridoma cell culture supernatant contains IgG between 5 - 100 µg/ml (Dilution factor 1:1000-1:10,000). Use Diluent Buffer to dilute your samples.
  Notes: Store samples on ice if they will be tested within 2 hours (freshly prepared samples yield the best results). For long-term storage, aliquot and freeze at -20°C (≤ 2 weeks) or -80°C (≤ 1 months). Avoid repeated freeze-thaw cycles. Bring samples to room temperature before the assay.

VIII. Assay Protocol:

Notes:
Preliminary experiments using standards and a small number of samples to optimize assay procedure and appropriate sample dilution is recommended. Carefully add samples and reagents without touching the well walls and mix gently without creating foam or bubbles to ensure sensitivity and consistency of generated data. Measure all standards and samples in duplicates for best results. The 96-well plate should not be dry at any time.

1. Wash wells with 100 µl **PBST**. Cover the plate and incubate at room temperature for 10 min.
2. Remove cover and discard buffer. Add 100 µl of all **standards** in duplicates in the first 2 rows of the plate.
3. Add 100 µl of diluted **samples** in duplicate to each empty well. Cover the plate and incubate at 37°C for 30 min.

4. Remove cover, discard solution and wash the plate 5 times with **PBST**. Wash by filling each well with 200 µl PBST using a multi-channel pipette or autowasher. Let it soak for 1-2 minutes, and remove all residual buffer by aspiration. After the last wash, remove any remaining PBST by aspirating or decanting. (Clap the plate on absorbent filter papers.)
5. Add 100 µl of **Detection antibody-HRP** into each well, cover the plate and incubate at 37°C for 30 min.
6. Discard the solution and wash 5 times with **PBST** as in step 4.
7. Add 100 µl of **TMB Substrate** into each well and incubate for 5 min at room temperature (avoid light).

   Note: The optimal incubation time can be determined by reading OD 650 nm. Different shades of blue can be seen in the wells proportional to the amount of mouse IgG in the standards and samples.
8. Add 50 µl **Stop Solution** into each well. The color will change into yellow immediately. Mix thoroughly by tapping the side of the plate without bubble formation.
9. Read absorbance at 450 nm in a microplate reader immediately after adding the stop solution for best result. The OD 450 nm should be about 2 times of OD 650 nm.

IX. Calculation:

Subtract zero standard from all readings. The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (X) vs. the respective concentration of the standard solution (Y). The mouse IgG concentration in the samples can be interpolated from the standard curve.

Mouse IgG Concentration = B X D (ng/ml) (B: Sample Concentration from the standard curve; D: Sample dilution factors)

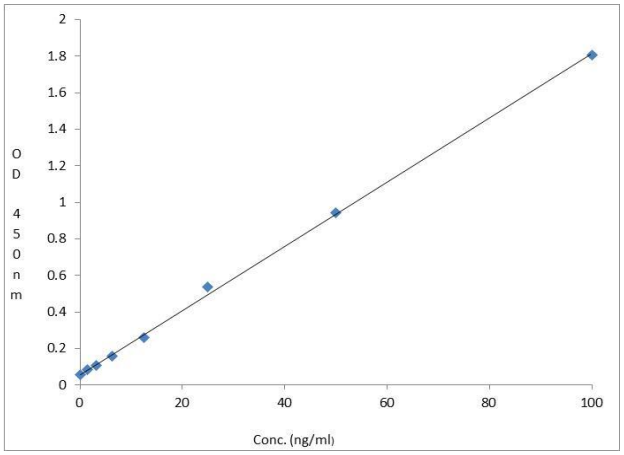


Figure: Typical Mouse IgG Standard Curve. This standard curves is for demonstration only. A standard curve must be run with each assay.

X. RELATED PRODUCTS:

Mouse IgG (1265-100, -1000)
Anti-Mouse IgG, HRP-Linked Antibody (6402-05)
Goat Anti-Mouse IgG (H&L) AMCA (6902-250)
TMB, High Kinetics (1216-100)
TMB, Ultrasensitive (1215-100)
Goat Anti-Mouse IgG (H&L) HRP (6920-100)
EZBlock™ T20 (PBS) Blocking Buffer (2143-1000)
Ready-to-use IHC/ICC kit (Biotin free), One-Step HRP Polymer anti-Mouse, Rat and Rabbit IgG (H+L) with DAB (K405-50)