ab285332 – Mouse IgG Quantification ELISA Kit

For quantitative measurement of total mouse IgG in serum, ascites, hybridoma cell culture supernatant samples, and other biological fluids.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab285332

Storage and Stability

The entire kit may be stored at -20°C, stable for 1 month after first use, if stored correctly.

Materials Supplied

Item	Quantity	Storage Condition
Pre-coated Microplate	8 x 12 wells	- 20°C
Mouse IgG Standard (100ng/ml)	500 µl	- 20°C
Detection antibody HRP	50 µl	- 20°C
Diluent buffer	35 ml	- 20°C
TMB substrate	10 ml	- 20°C
Stop Solution	11 ml	- 20°C
Plate sealers	2 units	- 20°C

Materials Required, Not Supplied

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

- 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

Reagent Preparation

Δ Note: *Before using the kit, spin tubes and bring down all components to the bottom of tubes. Bring all buffers and samples to room temperature (18-25°C) before starting.*

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.

Standard Preparation

1. To prepare 0.5 ml of 50 ng/ml standard, gently mix 250 µl of Standard stock solution in 250 µl of Diluent Buffer.
2. 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.
3. Use 250 µl Diluent Buffer as blank. The mouse IgG standard solutions are best used within 60 minutes on ice.

Sample Preparation

Δ Note: *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.*

- 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.

Assay Protocol

Δ Note: *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 auto washer. 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).

Δ 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

Measurement

Read absorbance at 450 nm within 20 minutes of adding stop solution. Subtract the absorbance of the blank from the readings for each standard and sample.

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)

Where: **B** = Sample Concentration from the standard curve
D = Sample dilution factors

Technical Support

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