Mouse IgG2a ELISA

INTRODUCTION

The mouse IgG2a ELISA kit is designed for measurement of IgG2a in mouse serum or plasma. The assay uses goat anti-mouse IgG for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated goat anti-mouse IgG2a antibodies for detection. The capture antibodies were cross-absorbed on mouse IgM and IgA agarose, and the detection antibodies were cross adsorbed on mouse IgM, IgA, IgG1, IgG2b and IgG3 agarose thereby ensuring specificity for IgG2a. Cross-reactivity with immunoglobulins from other species has not been investigated.

PRINCIPLE OF THE ASSAY

Test samples are diluted and incubated in the microtiter wells for 45 minutes alongside prepared mouse IgG2a standards. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 30 minutes. IgG2a molecules are thus sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRP-labeled antibodies and TMB Reagent is added and incubated for 20 minutes at room temperature. This results in the development of a blue color. Color development is stopped by the addition of Stop Solution, changing the color to yellow, and optical density is measured spectrophotometrically at 450 nm. The concentration of IgG2a is proportional to the optical density of the test sample and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti mouse IgG coated 96-well plate (12 strips of 8 wells)
- Anti mouse IgG2a-HRP Conjugate, 11 ml
- Reference standard (lyophilized)[1]
- 20x Wash Solution, 50 ml
- 10x Immunoglobulin Diluent, 25 ml
- TMB Reagent (One-Step), 11 ml
- Stop Solution (1N HCl), 11 ml

Materials required but not provided:

- Precision pipettes and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Micro-Plate incubator/shaker mixing speed of ~150 rpm
- Plate washer
- Plate reader with an optical density range of 0-4 at 450nm
- Graph paper (PC graphing software is optional)

[1] The reference standard consists of lyophilized mouse serum of known IgG2a concentration in a BSA matrix. The IgG2a concentration was determined relative to a mouse IgG2a standard obtained from an independent laboratory.

STORAGE

The test kit will remain stable for six months from the date of purchase provided that the components are stored at 2-8°C. The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air.

GENERAL INSTRUCTIONS

1. Please read and understand the instructions thoroughly before using the kit.
2. All reagents should be allowed to reach room temperature (18-25°C) before use.
3. Optimal results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.

IMMUNOGLOBULIN DILUENT PREPARATION

The diluent is provided as a 10x stock. Prior to use, estimate the final volume of diluent required for your assay and dilute one (1) volume of the 10x stock with nine (9) volumes of distilled or deionized water.

WASH SOLUTION PREPARATION

The wash solution is provided as a 20x stock. Prior to use, dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water.

STANDARD PREPARATION

1. The mouse IgG2a standard is provided as a lyophilized stock. Reconstitute as detailed on the vial label ***(the reconstituted standard is stable at 4°C for one week but should be aliquoted and frozen at -20°C after reconstitution if future use is intended)***.
2. Label 6 polypropylene or glass tubes as 6.25, 3.125, 1.563, 0.781, 0.391, and 0.195 ng/ml.
3. Into the tube labeled 6.25 ng/ml, pipette the volume of diluent detailed on the IgG2a standard vial label. Then add the indicated volume of IgG2a standard (shown on the IgG2a standard vial label) and mix gently. This provides the 6.25 ng/ml standard.
4. Dispense 250 µl of diluent into the tubes labeled 3.125, 1.563, 0.781, 0.391, and 0.195 ng/ml.
5. Prepare a 3.125 ng/ml standard by diluting and mixing 250 µl of the 6.25 ng/ml standard with 250 µl of diluent in the tube labeled 3.125 ng/ml.
6. Similarly prepare the 1.563, 0.781, 0.391, and 0.195 ng/ml standards by serial dilution.

SAMPLE PREPARATION

General Note: Mouse serum IgG2a concentrations vary with species and treatment conditions. In our laboratory we have measured values of 0.05 to 1.5 mg/ml or greater. We <u>suggest</u> an initial dilution factor of 100,000 fold using the following procedure. We also advise that a limited number of samples be tested initially (as singlets) in order to determine the optimal dilution factor.

1. Dispense 498 µl and 798 µl of 1x diluent into separate tubes.

2. Pipette and mix 2 µl of the serum/plasma sample into the tube containing 498 µl of diluent. This provides a 250 fold diluted sample.
3. Mix 2 µl of the 250 fold diluted sample with the 798 µl of diluent in the second tube. This provides a 100,000 fold dilution of the sample.
4. Repeat this procedure for each sample to be tested.

Tissue extracts and tissue culture medium will likely have lower IgG2a levels than those found in serum. Optimal dilutions of such samples should be determined empirically.

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards and diluted samples into the wells (we recommend that samples be tested in duplicate).
3. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 45 minutes.
4. Aspirate the contents of the microtiter wells and wash the wells 5 times with 1x wash solution using a plate washer (400 µl/well). The entire wash procedure should be performed as quickly as possible.
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual wash buffer.
6. Add 100 µl of enzyme conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 30 minutes.
8. Wash as detailed in 4 to 5 above.
9. Dispense 100 µl of TMB Reagent into each well.
10. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
11. Stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix. *It is important to make sure that all the blue color changes to yellow.*
13. Read the optical density at 450 nm with a microtiter plate reader *within 5 minutes*.

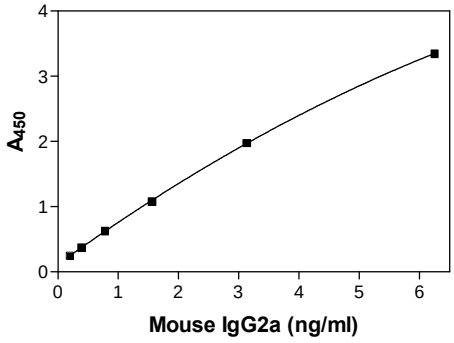
CALCULATION OF RESULTS

1. Calculate the average absorbance values (A_{450}) for each set of reference standards and samples.
2. Construct a standard curve by plotting the mean absorbance obtained from each reference standard against its concentration in ng/ml on linear graph paper, with absorbance values on the vertical or Y-axis and concentrations on the horizontal or X-axis.
3. Using the mean absorbance value for each sample, determine the corresponding concentration of IgG2a in ng/ml from the standard curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of IgG2a in the sample.
5. PC graphing software may be used for the above steps.
6. If the OD_{450} values of samples fall outside the standard curve, samples should be diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

A typical standard curve with optical density readings at 450nm on the Y-axis against IgG2a concentrations on the X-axis is shown below. This curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain his or her data and standard curve in each experiment.

IgG2a (ng/ml)	A_{450}
6.25	3.347
3.125	1.979
1.563	1.082
0.781	0.624
0.395	0.371
0.195	0.253



LIMITATIONS OF THE PROCEDURE

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of and in accordance with the instructions detailed above.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.

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For technical assistance please email us at
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