Version 24-04/25

User's Manual

Rat IgG1 ELISA Kit

REF **DEIA-BY043**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

This kit can be used for quantitative measurement of IgG1 in rat serum or plasma.

Principles of Testing

Test samples are diluted and incubated in the anti-rat IgG1 coated microtiter wells for 45 minutes alongside rat IgG1 standards. The microtiter wells are subsequently washed, and anti-rat IgG-HRP conjugate is added and incubated for 45 minutes. IgG1 molecules are thus sandwiched between the immobilization and detection antibodies. The wells are then washed to remove unbound HRPlabeled 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 IgG1 is proportional to the optical density of the test sample and is derived from a standard curve.

Reagents And Materials Provided

1. Anti-Rat IgG1 Coated 96-well Plate (12 strips of 8 wells)
2. Anti-Rat IgG HRP Conjugate Reagent, 11 ml
3. Reference Standard (lyophilized)
4. 20x Wash Solution, 50 ml
5. 10x Immunoglobulin diluent, 25 ml
6. TMB Reagent (One-Step), 11 ml
7. Stop Solution (1N HCl), 11 ml

Materials Required But Not Supplied

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

Storage

The test kit will remain stable for six months from the date of purchase provided that the components are stored at 4°C. The microtiter plate should be kept in a sealed bag with desiccant to minimize exposure to damp air.

Specimen Collection And Preparation

General Note: IgG1 is typically present in rat serum or plasma at concentrations of approximately 0.1 mg/ml. In order to obtain values within range of the standard curve, we suggest that samples initially be diluted 1000 fold using the following procedure for each sample to be tested:

1. Dispense 247.5 µl and 225 µl of 1x diluent into separate tubes.
2. Pipette and mix 2.5 µl of the serum/plasma sample into the tube containing 247.5 µl of diluent. This provides a 100 fold diluted sample.
3. Mix 25 µl of the 100 fold diluted sample with the 225 µl of diluent in the second tube. This provides a 1000 fold dilution of the sample.
4. Repeat this procedure for each sample to be tested.

Tissue extracts and body fluids other than serum or plasma will likely have lower IgG1 levels than those found in serum. Optimal dilutions of such samples should be determined empirically.

Reagent Preparation

1. All reagents should be allowed to reach room temperature (18- 25°C) before use.
2. Optimum results are achieved if, at each step, reagents are pipetted into the wells of the microtiter plate within 5 minutes.
3. 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.
4. 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.
5. Standard Preparation:

1) The rat IgG1 standard is provided as a lyophilized stock. Reconstitute with 1.0 ml of distilled or deionized water (the reconstituted standard is stable at 4°C for one day but should be aliquoted and frozen at -20°C after reconstitution if future use is intended).
2) Label 6 polypropylene or glass tubes as 500, 250, 125, 62.5, 31.25 and 15.63 ng/ml.
3) Into the tube labeled 500 ng/ml, pipette the volume of diluent detailed on the IgG1 standard vial label. Then add the indicated volume of IgG1 standard and mix gently. This provides the 500 ng/ml standard.
4) Dispense 250 µl of diluent into the tubes labeled 250, 125, 62.5, 31.25, 15.63 ng/ml.
5) Prepare a 250 ng/ml standard by diluting and mixing 250 µl of the 500 ng/ml standard with 250 µl of diluent in the tube labeled 250 ng/ml.
6) Similarly prepare the 125, 62.5, 31.25 and 15.63 ng/ml standards by serial dilution.

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 (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 HRP conjugate reagent into each well.
7. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (25°C) for 45 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 (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.

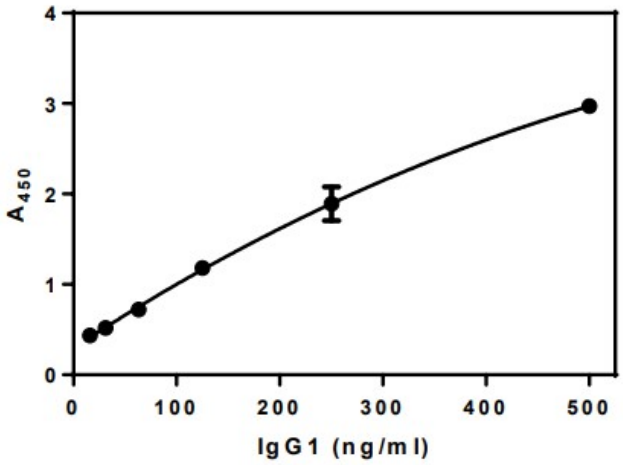
Calculation

1. Calculate the average absorbance values (A450) 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 IgG1 in ng/ml from the standard curve.
4. Multiply the derived concentration by the dilution factor to determine the actual concentration of IgG1 in the sample.
5. Ideally, PC graphing software may be used for the above steps. We find good fits of standard curve data to a one site – total and nonspecific binding model.
6. If the OD450 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 IgG1 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.

IgG1 (ng/ml)	A_{450}
500	2.971
250	1.891
125	1.181
62.5	0.723
31.25	0.519
15.63	0.434



Specificity

The kit recognizes only IgG1 in rat serum. It does not recognize mouse IgG. Cross-reactivity with immunoglobulins from other species has not been investigated.

Precautions

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.