RAT MURINOGLOBULIN ELISA
Life Diagnostics, Inc., Catalog Number: 2710-2

Enzyme Immunoassay for the Quantitative Determination of Rat Murinoglobulin in Serum or Plasma

INTRODUCTION

Murinoglobulin is a negative acute phase reactant, the levels of which decrease in rat serum or plasma as a result of injury, infection or disease. In a rat adjuvant induced arthritis model murinoglobulin levels decrease by approximately eight fold[1]. Murinoglobulin therefore provides an excellent marker of inflammation/disease in rats.

PRINCIPLE OF THE TEST

The rat murinoglobulin ELISA is based on a solid phase enzyme-linked immunosorbent assay (ELISA). The assay uses affinity purified anti-rat murinoglobulin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated anti-rat murinoglobulin antibodies for detection. The test sample is diluted and incubated in the microtiter wells for 45 minutes. The microtiter wells are subsequently washed and HRP conjugate is added and incubated for 30 minutes. This results in murinoglobulin molecules being 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 murinoglobulin is proportional to the optical density of the test sample.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Anti-rat murinoglobulin antibody coated microtiter plate with 96 wells (provided as 12 detachable strips of 8)
- Enzyme Conjugate Reagent, 11 ml
- Reference standard (1 vial, lyophilized) containing 2 μg/ml rat murinoglobulin when reconstituted as detailed on the vial label
- 10x Diluent (25 ml)
- 20x Wash solution (50 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 reader with an optical density range of 0-4 at 450 or 405 nm
- Graph paper (PC graphing software is optional).

STORAGE OF TEST KIT

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

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. Serum or plasma samples should be diluted ~100,000 fold with 1x diluent in order to obtain values within the standard range.

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 rat murinoglobulin standard is provided as a lyophilized stock. Add the volume of distilled or de-ionized water indicated on the vial label and mix gently until dissolved to obtain a 2 μg/ml rat murinoglobulin stock ***(the reconstituted standard remains stable for at least 2 days at 2-8°C but should be aliquoted and frozen at or below -20°C after reconstitution if use beyond this time is intended)***.
2. Label 7 polypropylene or glass tubes as 125, 62.5, 31.2, 15.6, 7.8, 3.9 and 0 ng/ml.
3. Dispense 937.5 μl of diluent into the tube labeled 125 ng/ml and 300 μl of diluent into the remaining tubes.
4. Pipette 62.5 μl of the 2 μg/ml murinoglobulin standard into the tube labeled 125 ng/ml and mix. This provides the working 125 ng/ml murinoglobulin standard.
5. Prepare a 62.5 ng/ml standard by diluting and mixing 300 μl of the 125 ng/ml standard with 300 μl of diluent in the tube labeled 62.5 ng/ml. Similarly prepare the 31.25, 15.6, 7.8, and 3.9 ng/ml standards by serial dilution.

SAMPLE PREPARATION

General Note: Murinoglobulin is present in normal rat serum at a concentration of ~ 6 mg/ml. In order to obtain values within the range of the standard curve, we suggest that samples be diluted 100,000 fold using the following procedure for each sample to be tested:

1. Dispense 998 μl and 497.5 μl of 1x diluent into separate tubes.

2. Pipette and mix 2 µl of the serum/plasma sample into the tube containing 998 µl of diluent. This provides a 500 fold diluted sample.
3. Mix 2.5 µl of the 500 fold diluted sample with the 497.5 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

ASSAY PROCEDURE

1. Secure the desired number of coated wells in the holder.
2. Dispense 100 µl of standards and 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. Remove the incubation mixture by flicking plate contents into an appropriate Bio-waste container.
5. Wash and empty the microtiter wells 5 times with 1x wash solution. This may performed using either a plate washer (350 µl/well) or with a squirt bottle. The entire wash procedure should be performed as quickly as possible.
6. Strike the wells sharply onto absorbent paper or paper towels to remove all residual water droplets.
7. Add 100 µl of enzyme conjugate reagent into each well.
8. Incubate on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 30 minutes.
9. Wash as detailed in 4 and 5 above.
10. Strike the wells sharply onto absorbent paper or paper towels to remove residual water droplets.
11. Dispense 100 µl of TMB Reagent into each well.
12. Gently mix on an orbital micro-plate shaker at 100-150 rpm at room temperature (18-25°C) for 20 minutes.
13. Stop the reaction by adding 100 µl of Stop Solution to each well.
14. Gently mix. It is important to make sure that all the blue color changes to yellow.
15. Read the optical density at 450 nm with a microtiter plate reader within 15 minutes. ***Please Note: If the A_{450} of the high standard(s) exceeds the limits of the plate reader, absorbance may be determined at 405 nm.***

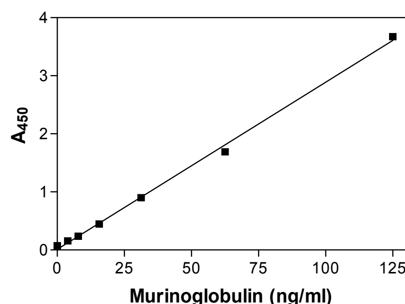
CALCULATION OF RESULTS

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 murinoglobulin in ng/ml from the standard curve.
4. Multiply the derived concentrations by the dilution factor to determine the actual concentration of murinoglobulin in the serum/plasma 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 when tested at a dilution of 100,000. 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 murinoglobulin 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.

Murinoglobulin (ng/ml)	Absorbance (450 nm)
125	3.673
62.5	1.689
31.25	0.899
15.63	0.446
7.81	0.235
3.95	0.156
0	0.072



REFERENCES

1. K Lonberg-Holm et.al., Three high molecular weight protease inhibitors of rat plasma: isolation, characterization, and acute phase changes. J Biol Chem. 262:438-445 (1987)