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

INTRODUCTION

Murinoglobulin is a negative acute phase reactant, the levels of which decrease in rat serum or plasma because of injury, infection or disease. In a rat adjuvant induced arthritis model, murinoglobulin levels decrease by approximately eight fold[1].

PRINCIPLE OF THE ASSAY

The assay uses affinity purified rat murinoglobulin antibodies for solid phase (microtiter wells) immobilization and horseradish peroxidase (HRP) conjugated rat murinoglobulin antibodies for detection. Standards and diluted samples are incubated in the microtiter wells for 45 minutes. The wells are subsequently washed. 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-conjugate and TMB is added and incubated for 20 minutes. If murinoglobulin is present a blue color develops. Color development is stopped by the addition of Stop solution, changing the color to yellow, and absorbance is measured at 450 nm. The concentration of murinoglobulin is proportional to absorbance and is derived from a standard curve.

MATERIALS AND COMPONENTS

Materials provided with the kit:

- Murinoglobulin antibody coated 96-well plate (12 x 8-well strips)
- HRP Conjugate, 11 ml
- Murinoglobulin stock (lyophilized)
- 20x Wash solution; TBS50-20, 50 ml
- 10x Diluent; YD25-10, 25 ml
- TMB, TMB11-1, 11 ml
- Stop solution, SS11-1, 11 ml

Materials required but not provided:

- Pipettors and tips
- Distilled or deionized water
- Polypropylene or glass tubes
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm.
- Curve fitting software

STORAGE

The unused kit should be stored at 2-8°C and the microtiter plate should be kept in a sealed bag with desiccant. Kits will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. Reliable and reproducible results will be obtained when the assay is carried out with a complete understanding of the instructions and with adherence to good laboratory practice.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. Our ELISA kits are calibrated using shaking incubators set at 150 rpm and 25°C. Performance of the assay at lower temperatures will result in lower absorbance values.

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 volume of the 10x stock with nine 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 stock is provided lyophilized. 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 solution *(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 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 1x diluent into the tube labeled 125 ng/ml and 300 μl of diluent into the remaining tubes.
4. Prepare the 125 ng/ml standard by mixing 62.5 μl of the 2 μg/ml with 937.5 μl of 1x diluent in the tube labeled 125 ng/ml.
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.
6. Similarly prepare the 31.25, 15.6, 7.8, and 3.9 ng/ml standards by serial dilution.

SAMPLE PREPARATION

Murinoglobulin is present in normal rat serum at a concentration of approximately 6 mg/ml. 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 2.0 μl of the serum/plasma sample into the tube containing 998 μl of diluent. This provides a 500-fold dilution.
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.

ASSAY PROCEDURE

1. Secure the desired number of 8-well strips in the holder. Unused strips should be stored in the re-sealed bag with desiccant at 2-8°C for future use.
2. Dispense 100 μl of standards and samples into the wells (we recommend that standards and samples be run in duplicate).
3. Incubate on an orbital micro-plate shaker at 150 rpm and 25°C for 45 minutes.
4. Empty and wash the microtiter wells 5x with 1x wash solution using a plate washer (400 μl/well).
5. Strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
6. Add 100 μl of HRP-conjugate into each well.

7. Incubate on a plate shaker at 150 rpm and 25°C for 30 minutes.
8. Wash as detailed above.
9. Strike the wells sharply onto absorbent paper or paper towels to remove residual droplets.
10. Dispense 100 µl of TMB into each well.
11. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
12. After 20-minutes, stop the reaction by adding 100 µl of Stop solution to each well.
13. Gently mix. It is important to make sure that all the blue color changes to yellow.
14. Read absorbance at 450 nm with a plate reader within 5 minutes.

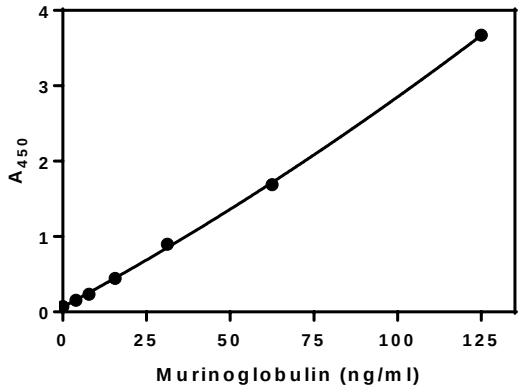
CALCULATION OF RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus concentration.
2. Fit the standard curve to an appropriate model and derive the concentration of the samples (we recommend using a single site, total and nonspecific binding model).
3. Multiply the derived concentration by the dilution factor to determine the actual concentration in the serum sample.
4. If the A_{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 absorbance at 450 nm on the Y-axis against murinoglobulin concentrations on the X-axis is shown below. This curve is for illustration only.

Murinoglobulin (ng/ml)	A_{450}
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)

Rev 121417

For technical assistance please email us at
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