



## Rat IgM ELISA Kit

Enzyme Immunoassay for the quantification of Rat IgM in Serum, plasma and cell culture supernatants.

Catalog number: ARG82298

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For research use only. Not for use in diagnostic procedures.

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## **INTRODUCTION**

Immunoglobulin M (IgM) is produced as a 900 kDa pentamer, which is an efficient complement binder. This antibody type is produced initially in the immune response and it is the first immunoglobulin class to be synthesized by a fetus or newborn. IgM antibodies do not cross the placenta. IgM concentration in blood is 0.12 g/l and its biological survival (plasma T<sub>1/2</sub>) is 5 days.

Mediates B-cell proliferation in the absence of co-stimulus as well as IgE production in the presence of IL-4. Involved in immunoglobulin class switching. Release of soluble CD40L from platelets is partially regulated by GP IIb/IIIa, actin polymerization, and an matrix metalloproteinases (MMP) inhibitor-sensitive pathway. [UniProt]

## **PRINCIPLE OF THE ASSAY**

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for IgM has been pre-coated onto a microtiter plate. Standards or samples are pipetted into the wells and any IgM present is bound by the immobilized antibody. After washing away any unbound substances, a HRP-conjugated antibody specific for IgM is added to each well and incubate. Following a washing to remove unbound substances, a substrate solution (TMB) is added to the wells and color develops in proportion to the amount of IgM bound in the initial step. The color development is stopped by the addition of acid and the intensity of the color is measured at a wavelength of 450nm ±2nm. The concentration of IgM in the sample is then determined by comparing the O.D of samples to the standard curve.

## **MATERIALS PROVIDED & STORAGE INFORMATION**

Store the unopened kit at 2-8 °C. Use the kit before expiration date.

<b>Component</b>	<b>Quantity</b>	<b>Storage information</b>
Antibody-coated microplate	8 X 12 strips	4°C. Unused strips should be sealed tightly in the air-tight pouch.
Standard (Lyophilized)	3 X 10 ng/vial	4°C
Standard diluent buffer	40 ml	4°C
Antibody conjugate concentrate	1 vial (400 µl)	4°C
Antibody diluent buffer	16 ml	4°C
20X Wash buffer	50 ml	4°C
TMB substrate	12 ml	4°C (Protect from light)
STOP solution	12 ml	4°C
Plate sealer	4 strips	Room temperature

## **MATERIALS REQUIRED BUT NOT PROVIDED**

- Microplate reader capable of measuring absorbance at 450nm
- Pipettes and pipette tips
- Deionized or distilled water
- 37°C oven or incubator
- Automated microplate washer (optional)

## **TECHNICAL HINTS AND PRECAUTIONS**

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Store the kit at 4°C at all times.
- If crystals are observed in the 20X Wash buffer, warm to RT (not more than 50°C) until the crystals are completely dissolved.

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- Ensure complete reconstitution and dilution of reagents prior to use.
- All materials should be equilibrated to room temperature (RT, 22-25°C) 20 min before use.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Mix the contents of the microplate wells thoroughly by microplate shaker for 1 min or gently tap the plate to ensure good test results. Please mix carefully to avoid well-to-well contamination. Do not reuse microwells.
- The TMB Color developing agent should be colorless and transparent before using.
- Use reservoirs only for single reagents. This especially applies to the substrate reservoirs. Using a reservoir for dispensing a substrate solution that had previously been used for the conjugate solution may turn solution colored. Do not pour reagents back into vials as reagent contamination may occur.
- Do not let wells dry during assay; add reagents immediately after completing the rinsing steps.
- Avoid using reagents from different batches.
- It is highly recommended that the standards, samples and controls be assayed in duplicates.
- Change pipette tips between the addition of different reagent or samples.

## **SAMPLE COLLECTION & STORAGE INFORMATION**

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

**Cell Culture Supernatants** - Remove particulates by centrifugation and aliquot & store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Serum** - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Collect serum and assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Plasma** - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

Note:

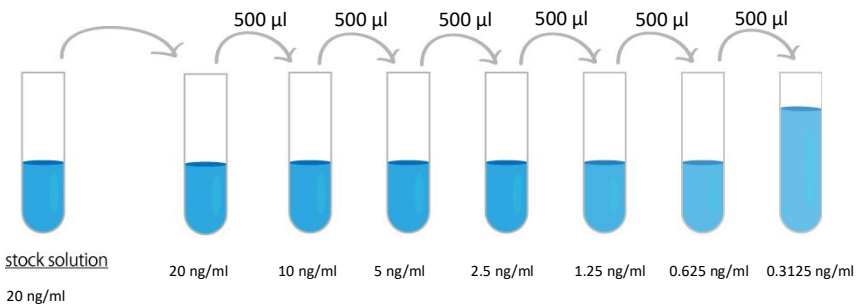
- a) Do not use haemolytic, icteric or lipaemic specimens.
- b) Samples containing sodium azide should not be used in the assay.

## REAGENT PREPARATION

- **1X Wash buffer:** Dilute 20X Wash buffer into distilled water to yield 1X Wash buffer. (E.g. 50 ml of 20X Wash buffer + 950 ml of distilled water)  
The diluted Wash buffer is stable for 4 weeks at 2°C to 8°C.
- **1X Antibody conjugate:** Dilute 30X antibody conjugate concentrate into 1X antibody diluent buffer to yield 1X Detection antibody solution.
- **Sample:** If the initial assay found samples contain IgM higher than the highest standard, the samples can be diluted with Standard diluent buffer and then re-assay the samples. For the calculation of the concentrations this dilution factor has to be taken into account. For normal mouse serum or plasma, user may dilute the samples starting with 1:50 dilution.

(It is recommended to do pre-test to determine the suitable dilution factor).

- **Standards:** Reconstitute the standard with 500 µl standard diluent buffer to yield a stock concentration of 20 ng/ml. Make sure the standard is dissolved completely before making serial dilutions. The standard diluent buffer serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: 20 ng/ml, 10 ng/ml, 5 ng/ml, 2.5 ng/ml, 1.25 ng/ml, 0.625 ng/ml, 0.3125 ng/ml.



## **ASSAY PROCEDURE**

All materials should be equilibrated to room temperature (RT) before use. Standards, samples and controls should be assayed in duplicates.

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal it.
2. Add 100  $\mu$ l of standards, samples and zero controls (standard diluent buffer) into wells. Incubate for 1.5 h at 37°C.
3. Aspirate each well and wash, repeating the process four times for a total five washes. Wash by filling each well with 1 $\times$  Wash Buffer (350  $\mu$ l) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add 100  $\mu$ l 1X Antibody conjugate into each well. Cover wells and incubate for 30 min at 37°C.
5. Aspirate each well and wash as step 3.
6. Add 100  $\mu$ l of TMB Reagent to each well, gently tap the plate to mix well. Incubate for 15 minutes at 37°C in dark.
7. Add 100  $\mu$ l of Stop Solution to each well, gently tap the plate to mix well. The color of the solution should change from blue to yellow.
8. Read the OD with a microplate reader at 450nm immediately. It is recommended read the absorbance within 3 min after adding STOP solution.

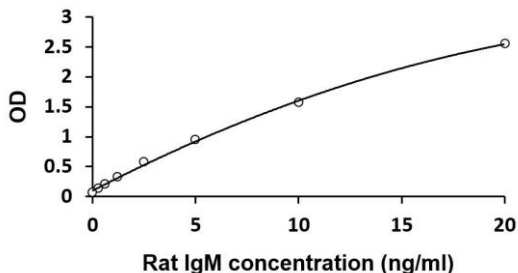


## **CALCULATION OF RESULTS**

1. Calculate the average absorbance values for each set of standards, controls and patient samples.
2. Using linear graph paper, construct a standard curve by plotting the mean absorbance obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Using the mean absorbance value for each sample determine the corresponding concentration from the standard curve.
4. Automated method: The results in the IFU have been calculated automatically using a 4 PL (4 Parameter Logistics) curve fit. 4 Parameter Logistics is the preferred method. Other data reduction functions may give slightly different results.
5. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure as described above.

### EXAMPLE OF TYPICAL STANDARD CURVE

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



### QUALITY ASSURANCE

#### Sensitivity

The minimum detectable dose (MDD) of Rat IgM ranged from 0.313- 20 ng/ml.

The mean MDD was 0.16 ng/ml.

#### Specificity

This assay recognizes natural and recombinant Rat IgM.

#### Intra-assay and Inter-assay precision

The CV values of both intra and inter precision fall below 10%.