Rat IgG ELISA

Catalog Number: 0801009

INTENDED USE

The IMMUNO-TEK Rat IgG ELISA* Kit is a rapid, easy to use enzyme linked immunosorbent assay (ELISA) designed for the measurement of rat IgG in rat serum, plasma, saliva, mucosa, cell culture fluid from rat cells or other biological sample. The assay contains ready-to-use reagents and takes less than two hours to perform. The microplate and detector antibody in the kit react with all subclasses of rat IgG.

*** Research Purposes Only. Not For *in vitro* Diagnostic Use.**

PRINCIPLE OF THE TEST

Microplate wells coated with polyclonal antibodies to rat IgG form the capture phase of the assay. Rat IgG specimen samples along with the supplied standard are diluted appropriately with Assay Diluent and are then incubated in the microplate wells. After a wash step, captured rat IgG in the well is incubated with detector antibody, a polyclonal anti-rat IgG conjugated to horseradish peroxidase (HRP). This antibody reacts only to the Fc portion of the immunoglobulin molecule allowing for the specific detection of IgG. After another wash step, the chromogenic substrate tetramethyl benzidine (TMB) is added and a blue color develops in proportion to the amount of rat IgG that has been bound to the antibody-coated plate. The enzyme reaction is stopped by the addition of Stop Solution which results in a color change to yellow which can be measured spectrophotometrically at 450 nm. The concentrations of rat IgG are then calculated from a standard curve.

REAGENTS

Materials Supplied:

- **Microplate, (1 x 96 well):** 12 x 8-well strips coated with goat anti-rat IgG
- **Rat IgG Detector Antibody (12 ml):** Contains HRP conjugated goat anti-rat IgG
- **Rat IgG Standard (7 ml):** Contains 125 ng/ml of rat IgG in Assay Diluent
- **Assay Diluent (100 ml):** Contains PBS, blocking proteins, Triton X-100® and 2-chloroacetamide as a preservative.
- **10X Plate Wash Buffer (125 ml):** Contains PBS, Tween 20® and 2-chloroacetamide as a preservative
- **Substrate (12 ml):** Contains Tetramethyl Benzidine (TMB)
- **Stop Solution (12 ml):** Proprietary formulation
- **Microplate Sealers (1 pk):** 10 sealers per pack
- **Sealable Plastic Bag (1 bag):** For storage of unused microplate strips

® Triton X-100 is a registered trademark of Union Carbide Chemicals and Plastics Co., Inc.
Tween 20 is a registered trademark of Imperial Chemical Industries.

This product was manufactured in a facility which has a Quality Management System that is ISO 13485 certified.

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878 Main Street
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800-274-5487
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Materials required but not supplied:

- Personal protection equipment (Disposable gloves, lab coat, safety glasses, etc.)
- Distilled or deionized water
- Graduated cylinders and beakers
- Test tubes and racks for preparing specimen and IgG standard dilutions
- Validated adjustable micropipettes and tips, single and/or multi-channel
- Timer
- Validated incubator or temperature controlled room at 18-25˚C
- Absorbent paper towels
- Validated microplate reader capable of measuring optical density at 450nm
- Automatic microplate washer or manual vacuum aspiration equipment
- Graph paper or computer graphing software

STORAGE

Store all kit reagents at 2-8°C. Do not freeze. Unused microplate strips should be kept in a sealed bag with desiccant to minimize exposure to moisture. All reagents stored and handled properly are stable at least until the expiration date printed on the kit box label.

PRECAUTIONS

- Prior to performing the assay, carefully read all instructions.
- Use universal precautions when handling kit components and test specimens.**
- To avoid cross-contamination, use separate pipette tips for each specimen.
- When testing potentially infectious specimens, adhere to all applicable local, state and federal regulations regarding the disposal of biohazardous materials.
- Stop Solution contains hydrochloric acid, which may cause severe burns. In case of contact with eyes or skin, rinse immediately with water and seek medical assistance. Wear protective clothing and eyewear.

**MMWR, June 24, 1988, Vol. 37, No. 24, pp. 377-382, 387-388

PREPARATION OF REAGENTS

Plate Wash Buffer:

Dilute 10X Plate Wash Buffer 1:10 in distilled or deionized water prior to use. Mix thoroughly. Prepared 1X Plate Wash Buffer can be stored at 2-8°C for up to one week. Additional 10X Plate Wash Buffer (ZMC Catalog #: 0801060) may be ordered if needed.

Rat IgG Standard Curve:

The Rat IgG Standard is provided at 125 ng/ml and should be diluted in Assay Diluent to prepare a standard curve as shown in the table below.

Tube Number	Concentration of Rat IgG	Volume of Rat IgG Standard	Volume of Assay Diluent
1	125 ng/ml	1000 µl	0 µl
2	62.5 ng/ml	500 µl of #1	500 µl
3	31.25 ng/ml	500 µl of #2	500 µl
4	15.6 ng/ml	500 µl of #3	500 µl
5	7.8 ng/ml	500 µl of #4	500 µl
6	0 ng/ml	0 µl	500 µl

Specimen Dilutions:

Rat sera or plasma have a range of 8-24 mg/ml of IgG. Because of this, we recommend preparing a 1:250,000-300,000-fold dilution in Assay Diluent for initial testing. To reduce dilution error, three dilution steps are recommended. For example, three 1:65-fold serial dilutions, where 10µl is added to 640µl of Assay Diluent, is a convenient dilution scheme. Other specimen samples may contain more or less IgG and will need to be diluted appropriately. A 10-fold dilution is usually sufficient to avoid possible matrix interference.

TEST PROCEDURE

Allow all reagents to reach room temperature before use. Prepare reagents as described above. Label test tubes to be used for the preparation of standards and specimen samples. If the entire microplate will not be used for testing, remove surplus strips from the plate frame and place into the sealable plastic bag with desiccant. Seal bag and store at 2-8°C.

Step 1: Label each strip on its end tab to ensure identity should the strips become detached from the plate frame during the assay.

Step 2: Pipette 200 µl of standards 1-6 into duplicate wells.

Step 3: Pipette 200 µl of each specimen sample into duplicate wells.

Step 4: Cover the microplate with a plate sealer and incubate for 30 minutes at room temperature (18-25˚C).

Step 5: Aspirate the contents of each well and wash 4 times with 1X Plate Wash Buffer (See Preparation of Reagents section). To wash, fill the wells with at least 300 µl of 1X plate wash buffer and aspirate. Perform 4 fill/aspirate cycles. After the final wash cycle, thoroughly blot the plate by carefully striking on a pad of absorbent paper towels. Continue striking until no visible droplets of Plate Wash Buffer are observed.

Step 6: Pipette 100 µl of Rat IgG Detector Antibody into each well.

Step 7: Cover the plate with a plate sealer and incubate for 30 minutes at room temperature (18-25˚C).

Step 8: Wash the plate 4 times with 1X Plate Wash Buffer as described in Step 5**.**

Step 9: Pipette 100 µl of Substrate into each well.

Step 10: Incubate the plate for 30 minutes at room temperature (18-25˚C) without a plate cover. A blue color will develop in wells containing rat IgG.

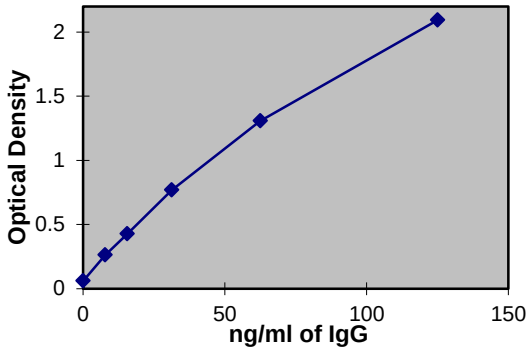
Step 11: Pipette 100 µl of Stop Solution into each well. A color change from blue to yellow will occur. Gently tap microplate to mix.

Step 12: Within 15 minutes, read the optical density of each well at 450 nm using a microplate reader.

EXPECTED RESULTS

Below is an example of a standard curve and should not be used to calculate actual samples. Variations may be observed from laboratory to laboratory due to pipetting, incubator temperatures, plate readers, etc.

Rat IgG Standard Concentration	Optical Density at 450 nm
125 ng/ml	2.093
62.5 ng/ml	1.307
31.25 ng/ml	0.769
15.6 ng/ml	0.427
7.8 ng/ml	0.262
0 ng/ml	0.065



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CALCULATION OF RESULTS

Test Validity:

- The mean optical density of the 0 ng/ml standard must be below 0.200.
- The mean optical density of the 125 ng/ml must be above 1.000.
- It is recommended that specimen samples be re-assayed with a greater or lesser dilution when the mean optical density value does not fall within the standard curve.

Calculation:

1. Calculate the mean optical density (OD) values for each set of standards and diluted specimen samples.
2. Using linear graph paper or graphing software, plot the mean OD values of each standard on the Y-axis versus the corresponding concentration of standards on the X-axis.
3. Draw the best fit curve through these points to construct a standard curve. A point-to-point construction will be the most accurate.
4. Determine the IgG concentration of each diluted specimen sample by interpolation from the standard curve.
5. Multiply by the dilution factor used to dilute each specimen sample to determine the IgG concentration of the original specimen sample.

PERFORMANCE CHARACTERISTICS

Specificity:

The rat IgG detector antibody in this kit has been shown by immunoelectrophoresis and/or ELISA to react only with the Fc portion of the Rat IgG heavy chain but not with the Fab portion of rat immunoglobulins. No reaction to other immunoglobulin classes (IgA, IgM, etc.) or other non-immunoglobulin serum proteins were detected.

Serum from Sheep, Rabbit, Chicken, Donkey, Goat, Horse, Bovine and Human had no reaction with this ELISA test. However, mouse serum does react even when IgG levels as low as 20 ng/ml are present.

Precision:

Sample	IgG ng/ml	Intra-assay %CV (n=16)	Inter-assay %CV (n=48)
High Sample	119	2.6%	4.3%
Medium Sample	29	2.4%	4.9%
Low Sample	7	3.3%	7.5%

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PROCEDURAL FLOW CHART

PREPARE REAGENT DILUTIONS

↓

PIPETTE SPECIMENS AND STANDARDS

↓

INCUBATE 30 MINUTES AT ROOM TEMPERATURE

↓

WASH PLATE

↓

PIPETTE DETECTOR ANTIBODY

↓

INCUBATE 30 MINUTES AT ROOM TEMPERATURE

↓

WASH PLATE

↓

PIPETTE SUBSTRATE

↓

INCUBATE 30 MINUTES AT ROOM TEMPERATURE

↓

ADD STOP SOLUTION AND READ AT 450 NM

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