

# Mouse IgG ELISA

## Catalog# 0801180

### INTENDED USE

The IMMUNO-TEK Mouse IgG ELISA Kit is a rapid, easy to use enzyme-linked immunosorbent assay (ELISA) designed for the measurement of mouse IgG in plasma, serum, hybridoma cell supernatants, ascites or other mouse biological fluids. The kit is especially useful in monitoring the production and purification of mouse monoclonal antibodies. The assay contains ready-to-use reagents and takes less than two hours to perform. The microplate and detector antibody in the kit have been specifically balanced to react uniformly with all subclasses of mouse IgG.

**The IMMUNO-TEK Mouse IgG ELISA Kit is for Research Purposes Only.**

### PRINCIPLE OF THE TEST

Microwells coated with polyclonal antibodies to mouse IgG form the capture phase of the assay. These antibodies bind uniformly to all subclasses of mouse IgG. Captured mouse IgG then reacts with detector antibody which is a polyclonal anti-mouse IgG conjugated with horseradish peroxidase. This reagent also reacts uniformly with all subclasses of mouse IgG. Enzyme activity in the wells are then quantified using tetramethyl benzidine as a substrate.

### REAGENTS

#### Materials Supplied:

- **Microplate, (1x96 well):** Strips coated with purified goat anti-mouse IgG
- **Detector Antibody (12 ml):** Contains conjugated goat anti-mouse IgG peroxidase
- **Mouse IgG Standard (7 ml):** Contains mouse IgG and assay diluent
- **Assay Diluent (100 ml):** Contains PBS, Triton X-100® and 2-chloroacetamide
- **Plate Wash Buffer (125 ml):** Contains PBS, Tween 20® and 2-chloroacetamide
- **Substrate (12 ml):** Contains Tetramethyl Benzidine (TMB)
- **Stop Solution (12 ml):** Proprietary formulation
- **Microtiter Plate Sealers (1 pk):** 10 sealers per pack
- **Plastic Bag (1 bag):** For storage of unused microtiter plate 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.

**Storage:**

Store all kit reagents at 2-8°C. Do not freeze.

**Materials Required but not Supplied:**

- Disposable gloves
- Test tubes and racks for preparing specimen and IgG standard dilutions
- Validated adjustable micropipettes, single and multi-channel
- Distilled or deionized water
- Validated incubator capable of maintaining 37 + 1 C
- Graduated cylinders and assorted beakers
- Validated microtiter plate reader
- Automatic microtiter plate washer or manual vacuum aspiration equipment
- Timer

**PRECAUTIONS**

**FOR RESEARCH USE ONLY. Not For *in vitro* Diagnostic Use.**

- 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.

**Mouse IgG Standard Curve**

Label 6 test tubes as shown below. The Mouse IgG Standard is provided at 125 ng/ml. This should be diluted in Assay Diluent as follows to prepare a standard curve.

Tube Number	Concentration of Mouse IgG	Volume of Mouse IgG Standard	Volume of Assay Diluent
1	125 ng/ml	1000 $\mu$ l	0 $\mu$ l
2	62.5 ng/ml	500 $\mu$ l of #1	500 $\mu$ l
3	31.25 ng/ml	500 $\mu$ l of #2	500 $\mu$ l
4	15.6 ng/ml	500 $\mu$ l of #3	500 $\mu$ l
5	7.8 ng/ml	500 $\mu$ l of #4	500 $\mu$ l
6	0 ng/ml	0 $\mu$ l	500 $\mu$ l

## **SPECIMEN DILUTIONS**

### **Serum and Plasma:**

Mouse serum and plasma samples typically contain 7-10 mg/ml of IgG. Therefore, we recommend preparing a 1:250,000 dilution of the sample in Assay Diluent for initial testing.

After initial testing, it may be necessary to adjust the concentration of the antibody solution to be tested in order to obtain a concentration between 125 ng/ml and 7.8 ng/ml for accurate quantification.

### **Hybridoma Supernatants:**

Hybridoma supernatants from stationary cell cultures will typically contain between 1 g/ml and 30 g/ml of monoclonal antibody. Therefore, we recommend preparing a 1:250 dilution of cell culture supernatants in Assay Diluent for initial testing.

When using cell culture supernatants from bioreactors, a further dilution may be necessary since many bioreactors are capable of producing much higher concentrations of monoclonal antibodies than standard stationary cell cultures. Refer to the technical literature provided with the bioreactor to determine a dilution that will yield a monoclonal antibody concentration between 125 ng/ml and 7.8 ng/ml.

After initial testing, it may be necessary to adjust the concentration of the antibody solution to be tested in order to obtain a concentration between 125 ng/ml and 7.8 ng/ml for accurate quantification.

### **Ascites:**

Mouse ascites fluid will typically contain between 1 mg/ml and 10 mg/ml of monoclonal antibody. Because of this, we recommend preparing a 1:100,000 dilution of ascites in Assay Diluent for initial testing.

After initial testing, it may be necessary to adjust the concentration of the antibody solution to be tested in order to obtain a concentration between 125 ng/ml and 7.8 ng/ml for accurate quantification.

## **TEST PROCEDURE**

Allow all reagents to reach room temperature before use. Label test tubes to be used for the preparation of standards and specimens. If the entire 96-well plate will not be used, remove surplus strips from the plate frame and place into the resealable 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:** Designate one well on the plate and leave empty. This well will serve as a substrate blank.

**Step 3:** Pipette 200  $\mu$ l of standards #1-6 into duplicate wells.

**Step 4:** Pipette 200  $\mu$ l of each specimen into duplicate wells.

**Step 5:** Cover the microplate with a plate sealer and incubate the plate for 30 minutes at 37 C.

**Step 6:** Aspirate the contents of each well and wash the wells 4 times with 1X Plate Wash Buffer. To wash, fill the wells with 300  $\mu$ l of 1X plate wash buffer and aspirate. Perform 4 fill/aspirate cycles. After the final wash cycle, thoroughly blot the plate by carefully striking the plate on a pad of absorbent paper towels. Continue until no visible droplets of Plate Wash Buffer are observed.

**Step 7:** Pipette 100  $\mu$ l of Detector Antibody into each standard and specimen well.  
**Do not add Detector Antibody to the substrate blank well.**

**Step 8:** Cover the plate with a plate sealer and incubate for 30 minutes at 37 C.

**Step 9:** Wash the plate 4 times with Plate Wash Buffer as described in **Step 6**.

**Step 10:** Pipette 100  $\mu$ l of Substrate into each well **including** the substrate blank well.

**Step 11:** Incubate the plate for 30 minutes at room temperature. A blue color will develop in wells containing mouse IgG.

**Step 12:** Pipette 100  $\mu$ l of Stop Solution into each well. A color change from blue to yellow will occur.

**Step 13:** Within 15 minutes, read the optical density of each well at **450 nm** using a microtiter plate reader.

### **Test Validity:**

For the test to be valid, the mean optical density of the 0 ng/ml standard and the substrate blank must be below 0.200.

## CALCULATION AND INTERPRETATION OF RESULTS

Using linear graph paper or a computer program, plot the optical densities of each standard on the Y-axis versus the corresponding concentration of the standards on the X-axis.

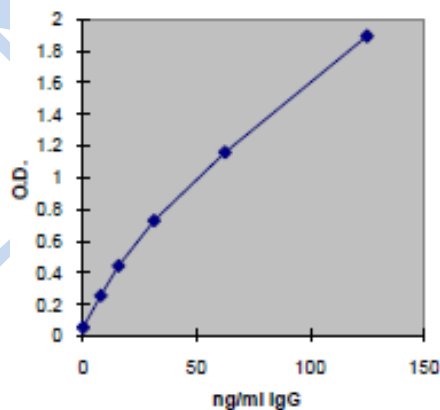
The concentration of mouse IgG in each diluted specimen may then be determined manually using a ruler to extrapolate, by linear regression using a computer program or pocket calculator with a linear regression function, or by point to point calculation again using a computer or calculator.

Correct the diluted specimen values by the dilution factor used to obtain the final concentration of Mouse IgG in the original specimen.

### **Typical Standard Curve**

Below is an example of a typical standard curve. Variations will occur from laboratory to laboratory due to pipetting, incubator temperatures, plate readers, etc.

Mouse IgG Standard Concentration	Optical Density at 450 nm
125 ng/ml	1.894
62.5 ng/ml	1.160
31.25 ng/ml	0.728
15.6 ng/ml	0.442
7.8 ng/ml	0.255
0 ng/ml	0.053
Substrate Blank	0.048



## PROCEDURAL FLOW CHART

PREPARE REAGENT DILUTIONS



PIPETTE SPECIMENS AND STANDARDS



INCUBATE 30 MINUTES AT 37° + 1°C



WASH PLATE



PIPETTE DETECTOR ANTIBODY



INCUBATE 30 MINUTES AT 37° + 1°C



WASH PLATE



PIPETTE SUBSTRATE SOLUTION



INCUBATE 30 MINUTES AT ROOM TEMPERATURE



ADD STOP SOLUTION AND READ AT 450 NM

For Research Use Only NOT for *in vitro* Diagnostic Use