Horse IgG ELISA

Catalog Number: 0801221

INTENDED USE

The IMMUNO-TEK Horse IgG ELISA Kit is a rapid, easy to use enzyme linked immunosorbent assay (ELISA) designed for the measurement of horse IgG in horse serum, plasma or other biological fluids. 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 horse IgG.

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

PRINCIPLE OF THE TEST

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

REAGENTS

Materials Supplied:

- **Microplate, (1x96 well):** Strips coated with purified goat anti-horse IgG
- **Detector Antibody (12 ml):** Contains conjugated goat anti-horse IgG peroxidase
- **Horse IgG Standard (7 ml):** Contains horse 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
- **Microplate Sealers (1 pk):** 10 sealers per pack
- **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.

REF	Catalog Number		Temperature Limitation
LOT	Lot Number		Expiration Date
RUO	For Research Use Only		

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878 Main Street
Buffalo, NY 14202
800-274-5487
www.zeptometrix.com

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°C± 1°C
- Graduated cylinders and assorted beakers
- Validated microtiter plate reader
- Automatic microtiter plate washer or manual vacuum aspiration equipment
- Timer

STORAGE

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

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.

Horse IgG Standard Curve

Label 6 test tubes as shown below. The Horse 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 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

Horse Serum

Horse serum will normally contain 10-15 mg/ml of IgG antibody. Because of this, we recommend preparing a 1:250,000 dilution of horse serum 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 µl of standards 1-6 into duplicate wells.

Step 4: Pipette 200 µl 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 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 the plate on a pad of absorbent paper towels. Continue until no visible droplets of Plate Wash Buffer are observed.

Step 7: Pipette 100 µl 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 µl of Substrate into each well **including** the substrate blank well.

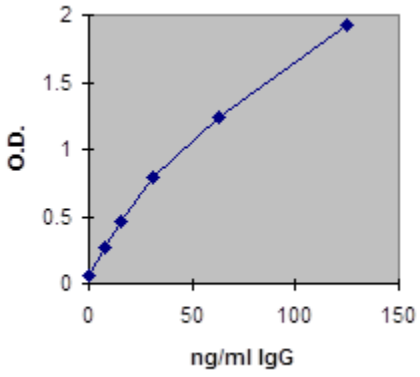
Step 11: Incubate the plate for 30 minutes at room temperature. A blue color will develop in wells containing horse IgG.

Step 12: Pipette 100 µl of Stop Solution into each well. A color change from blue to yellow will occur.

EXPECTED RESULTS

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

Horse 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
Substrate Blank	0.055



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CALCULATION OF RESULTS

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:

1. 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.

2. The concentration of horse 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.

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

PROCEDURAL FLOW CHART

PREPARE REAGENT DILUTIONS

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PIPETTE SPECIMENS AND STANDARDS

↓

INCUBATE 30 MINUTES AT 37º + 1ºC

↓

WASH PLATE

↓

PIPETTE DETECTOR ANTIBODY

↓

INCUBATE 30 MINUTES AT 37º + 1ºC

↓

WASH PLATE

↓

PIPETTE SUBSTRATE SOLUTION

↓

INCUBATE 30 MINUTES AT ROOM TEMPERATURE

↓

ADD STOP SOLUTION AND READ AT 450 NM

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