# Human Immunoglobulin G2 (IgG2)ELISA Kit

**Catalog No. CSB-E17026h**

(96T)

- This immunoassay kit allows for the in vitro quantitative determination of **human IgG2** concentrations in **serum, plasma .**
- **Expiration date** **six** months from the date of manufacture
- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

## PRINCIPLE OF THE ASSAY

The microtiter plate provided in this kit has been pre-coated with an antibody specific to IgG2. Standards or samples are then added to the appropriate microtiter plate wells with a HRP-conjugated antibody preparation specific for IgG2 to each microplate well and incubated. Then a TMB (3,3',5,5' tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain IgG2, HRP-conjugated antibody will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of IgG2 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

## DETECTION RANGE

0.48 ng/ml-2000 ng/ml. The standard curve concentrations used for the ELISA's were 2000 ng/ml,500 ng/ml,125 ng/ml, 31.25 ng/ml,7.81 ng/ml, 1.95 ng/ml, 0.48 ng/ml.

## SPECIFICITY

This assay recognizes human IgG2. No significant cross-reactivity or interference was observed.

## SENSITIVITY

The minimum detectable dose of human IgG2 is typically less than 0.48ng/ml.

The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest protein concentration that could be differentiated from zero

## MATERIALS PROVIDED

| Reagent | Quantity |
|---|---|
| Assay plate | 1 |
| Standard | 2 x 0.5 ml<br>(A solution of 2000 ng/ml) |
| Sample Diluent | 2 x 20 ml |
| HRP-conjugate | 1 x 120μl |
| HRP-conjugate Diluent | 1 x 20 ml |
| Wash Buffer | 1 x 20 ml<br>(25×concentrate) |
| TMB Substrate | 1 x 10 ml |
| Stop Solution | 1 x 10 ml |

## STORAGE

1. Unopened test kits should be stored at 2-8°C upon receipt and the microtiter plate should be kept in a sealed bag with desiccants to minimize exposure to damp air. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. A microtiter plate reader with a bandwidth of 10 nm or less and an optical density range of 0-3 OD or greater at 450nm wavelength is acceptable for use in absorbance measurement.

## REAGENT PREPARATION

***Bring all reagents to room temperature before use for 30min.***

1. **Wash Buffer** If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 500 ml of Wash Buffer.

2. **HRP-conjugate** Dilute to the working concentration using **HRP-conjugate Diluent**(1:100), respectively. The suggested 100-fold dilution can be achieved by adding 10 uL **HRP-conjugate** to 990uL of **HRP-conjugate Diluent** for 1ml working solution.

3. **Standard**

- Centrifuge the vial before opening. Allow the 2000 ng/ml standard solution to warm to room temperature . The 2000 ng/ml standard serves as the high standard**(tube#1)**. Sample Diluent serves as the zero standard(0ng /ml)**(tube#8)**.Label seven tubes#2 through #8.
- Pipette 150μl Sample Diluent into tubes#2-8. Add 150μl of the 2000ng/ml standard（tube #1）to tube #2. Vortex thoroughly. Add 150μl of tube #2 to tube #3 and vortex thoroughly,Continue this for tubes #3 through #7. Mix each tube thoroughly before the next transfer.

The concentration of **human IgG2** in tubes #1through #8 will be 2000,500,125, 31.25 ,7.81, 1.95, 0.48 and 0ng/ml respectively.

**Diluted standards should be used within 30 minutes of preparation.**

***Precaution: The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.***

## OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer

## SAMPLE COLLECTION AND STORAGE

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

**Note: Grossly hemolyzed samples are not suitable for use in this assay.**

## SAMPLE PREPARTION

Recommend to dilute the serum or plasma samples with Sample Diluent(1:5000) before test. The suggested 5000-fold dilution can be achieved by adding 2μl sample to 98μl of Sample Diluent, Complete the 5000-fold dilution by adding 2μl of this solution to 198μl of Sample Diluent. The recommended dilution factor is for reference only. The optimal dilution factor should be determined by users according to their particular experiments.

## ASSAY PROCEDURE

***Bring all reagents and samples to room temperature before use. It is recommended that all samples, standards, and controls be assayed in duplicate. All the reagents should be added directly to the liquid level in the well. The pipette should avoid contacting the inner wall of the well.***

1. Add 100μl of Standard or Sample per well. Cover with the adhesive strip. Incubate for 120min at 37°C.
2. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (200μl)

using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

3. Add 100µl of **HRP-conjugate working solution** to each well. Incubate for 60min at 37°C. **HRP-conjugate working solution** may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.
4. Repeat the aspiration and wash five times as before.
5. Add 90µl of **TMB Substrate** to each well. Incubate for 20 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.
6. Add 50µl of **Stop Solution** to each well when the first four wells containing the highest concentration of standards develop obvious blue color. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.

# CALCULATION OF RESULTS

***Using the professional soft "Curve Exert 1.3" to make a standard curve is recommended, which can be downloaded from our web.***

Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the

IgG2 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

## LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.

- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

## TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.