Human Rabies Virus Antibody (IgG) ELISA Kit

Catalog NO. CSB-E09547h
(96 tests)

- This immunoassay kit allows for the in vitro semi-quantitative determination of human rabies virus antibody (IgG) 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 purified rabies virus antigen. Samples are then added to the appropriate microtiter plate wells and incubated. Then add Horseradish Peroxidase (HRP)-conjugated anti-human IgG to each well and incubate. Finally, substrate solution is added to each well. 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. Calculate the valence of human rabies virus antibody(IgG) in the samples.

**SPECIFICITY**

This assay recognizes human rabies virus antibody(IgG). No significant cross-reactivity or interference was observed.
MATERIALS PROVIDED

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Assay plate</td>
<td>1</td>
</tr>
<tr>
<td>Negative Control</td>
<td>1x 0.5 ml</td>
</tr>
<tr>
<td>Positive Control</td>
<td>1x 0.5 ml</td>
</tr>
<tr>
<td>Sample Diluent</td>
<td>1 x 10 ml</td>
</tr>
<tr>
<td>HRP-conjugate</td>
<td>1 x 10ml</td>
</tr>
<tr>
<td>Wash Buffer</td>
<td>2 x 15 ml (20×concentrate)</td>
</tr>
<tr>
<td>Substrate A</td>
<td>1 x 5 ml</td>
</tr>
<tr>
<td>Substrate B</td>
<td>1 x 5 ml</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>1 x 5 ml</td>
</tr>
</tbody>
</table>

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. 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.
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 g. Remove serum and assay immediately or aliquot and store samples at -20° C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

- **Plasma** Collect plasma using citrate, EDTA, or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

*Note: Grossly hemolyzed samples are not suitable for use in this assay.*
**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 15 ml of Wash Buffer Concentrate into deionized or distilled water to prepare 300 ml of Wash Buffer.

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

**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. Set one Blank well, one Negative Control and one Positive Control.

2. Add 100μl of Sample Diluent to Blank well.

3. Add 100μl of Negative Control, Positive Control.

4. Add 100μl of Sample Diluent to each well for samples. Add 5μl Sample per well. Incubate for 30 min at 37°C.
5. Aspirate each well and wash, repeating the process five times for a total of six washes. Wash: Fill each well with Wash Buffer (300μl) and let it stand for 1 minutes, then remove the liquid by flicking the plate over a sink. The remaining drops are removed by patting the plate on a paper towel. Complete removal of liquid at each step is essential to good performance.

6. Add 100μl of *HRP-conjugate* to each well (not to blank well). Incubate for 30 minutes at 37°C. *HRP-conjugate* may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.

7. Wash plate six times as before.

8. Add 50μl of *Substrate A* and 50μl of *Substrate B* to each well. Incubate for 15 minutes at 37°C. Keeping the plate away from drafts and other temperature fluctuations in the dark.

9. Add 50μl of *Stop Solution* to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm.
CALCULATION OF RESULTS

For calculation the valence of human rabies virus antibody(IgG), compare the sample well with control.

When $\text{OD}_{\text{negative}} \leq 0.1$, the experiment result is credible.

While $\text{OD}_{\text{sample}} \geq \text{OD}_{\text{positive}}$: Positive

While $\text{OD}_{\text{sample}} < \text{OD}_{\text{positive}}$: Negative

(the value of neutralizing antibody for Rabies virus in the positive control is 0.5IU/ml)

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 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.