Mouse Anti-OVA IgG ELISA Kit

*Cat.No: DEIA-XY2117
Lot. No. (See product label)*

**Size**

96T

**Intended use**

CD provides this mouse anti-OVA IgG ELISA Kit for determining anti-ovalbumin (OVA) IgG antibody levels in mouse serum for studying the contribution of IgG in allergic diseases.

**Reagents And Materials Provided**

1. Standard Mouse IgG (Clone L-71 - Dr. Shin Yoshino of Kobe Pharmaceutical University): 1 vial, 1000 ng, lyophilized, -20°C
2. Ovalbumin (OVA): 1 vial, 100 µg, lyophilized, -20°C
3. Detection Antibody (Peroxidase-Conjugated Goat Anti-Mouse IgG Polyclonal Antibody): 2 vials, 1 µg, lyophilized, -20°C
4. Solution A - OVA Dilution Buffer: 1 bottle, 10 ml, -20°C
5. Solution B - Blocking Buffer: 1 bottle, 10 ml, -20°C
7. TMB Solution (contains DMSO): 2 vials, 0.2 ml, -20°C
8. Chromagen Dilution Buffer: 1 bottle, 20 ml, -20°C
9. Stop Solution - 2N Sulfuric Acid: 1 bottle, 10 ml, -20°C
10. Wash Buffer, 20X: 1 bottle, 50 ml, -20°C
11. ELISA Plate: 1 each, 96-well (8-well strips x 12), -20°C

**Assay Procedure**

1. Add OVA Solution: Dissolve one vial of Ovalbumin (OVA) with 10 ml of OVA Dilution Buffer (Solution A). Add 100 µl of OVA solution to each well and incubate at 4°C overnight.
2. Dilute Wash Buffer: Dilute 50 ml of 20X wash buffer in 950 ml of distilled water (1X wash buffer). Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. Do not allow the plate to dry out.
3. Add Blocking Buffer: Add 100 µl of the Blocking Buffer (Solution B) to each well and incubate at room temperature for 1 hour.
4. Prepare Standard Dilutions: The recommended standard range is 0.2-12.5 ng/ml. Dissolve one vial of Standard (IgG: 1,000 ng/vial) in 1 ml of Sample/Standard/Detection Antibody Dilution Buffer (Solution C). Take 25 µl of the standard solution and add to 1975 µl of Solution C to make 12.5 ng/ml of IgG solution. Then, serially dilute it with Solution C. For example, mix 250 µl of the standard (25 ng/ml) with an equal volume of Solution C to make 6.25 ng/ml solution, and then repeat it five more times for 3.125, 1.6, 0.8, 0.4 and 0.2 ng/ml standards.
5. Prepare Sample Dilutions: The dilution of serum from mouse immunized with OVA varies from 1:10 or more depending on the immunization schedule and timing of serum collection. In general, no IgG antibody against OVA is determined in normal serum at 1:10 dilution.
6. Wash: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. Do not allow the plate to dry out.
7. Add Standards and Samples: Add 100 µl of standards, Solution C (blank) and samples to wells in duplicate. Incubate at room temperature for 2 hours or at 4°C overnight. (OD values may be higher if standards and samples are incubated overnight at 4°C.)

8. Wash: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. Do not allow the plate to dry out.


10. Wash: Wash the plate with 1X wash buffer at least 3 times using a wash bottle with manifold or an automated plate washer. Empty the plate by inverting it and blot on a paper towel to remove excess liquid. Do not allow the plate to dry out.

11. Add TMB: Dilute one vial of TMB with 10 ml of Chromagen Dilution Buffer just prior to use. Add 100 µl of TMB solution to each well immediately after washing the plate and incubate for 25 minutes at room temperature.

12. Stop: Stop the reaction by adding 50 µl of 2N sulfuric acid (Stop Solution) to each well.

13. Read Plate: Read the OD values at 450 nm. If the OD values of samples are greater than the OD values of the highest standard, re-assay the samples at a higher dilution. A 630 nm filter can be used as a reference.

### Typical Standard Curve

A typical standard curve for mouse anti-OVA IgG ELISA Kit

![Typical Standard Curve](image-url)
Reproducibility

Reproducibility of data assayed by Mouse Anti-OVA IgG Antibody Assay Kit

<table>
<thead>
<tr>
<th>Test At</th>
<th>2.1 ng/ml</th>
<th>4 ng/ml</th>
<th>8.8 ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inter-Assay CV (%)</td>
<td>3.7</td>
<td>0.4</td>
<td>3.3</td>
</tr>
<tr>
<td>Intra-Assay CV (%)</td>
<td>9.5</td>
<td>5.2</td>
<td>2.6</td>
</tr>
<tr>
<td>Spiking Test*</td>
<td>0.4 ng/ml - 107%</td>
<td>1.8 ng/ml - 95%</td>
<td>6.3 ng/ml - 93%</td>
</tr>
</tbody>
</table>

A pooled normal mouse serum was added with known amounts of IgG, and then diluted with Sample/Standard/Detection Antibody Dilution Buffer for assaying anti-OVA IgG antibody by ELISA.

Precautions

1. It is recommended that the standard and samples be run in duplicate.
2. Partially used reagents may be kept at -20°C.
3. Crystals may form in the 20X wash buffer when stored at cold temperatures. If crystals have formed, it is necessary to warm the wash buffer by placing the bottle in warm water until crystals have dissolved completely.
4. Measure exact volume of buffers using a serological pipette prior to diluting. Extra buffer is provided.
5. Serum IgG antibodies are a mixture of multiple antibodies with a variety of affinity ranges. The OD value obtained in ELISA for antibody assay depends on the concentration of antibody as well as the affinity of antibody with an antigen. In general, ELISA data using a monoclonal antibody as a standard does not reflect the absolute levels of IgG. Therefore, IgG level determined by this kit should be expressed as ng of IgG equivalent to L-71 per ml.

References