Rabbit IgG ELISA

Catalog Number:
IGG79-K01 (1 x 96 wells)

For Research Use Only. Not for use in diagnostic procedures.

v. 1.0
Introduction
The Eagle Biosciences Rabbit IgG ELISA Assay Kit is designed for the quantitative determination of rabbit IgG in complex samples (serum or other biological samples). The Rabbit IgG ELISA Assay Kit is for research use only and should not be used in diagnostic procedures.

Principle of the Assay
The determination of rabbit IgG is carried out as direct sandwich ELISA. An antibody specific for rabbit IgG has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IgG present is bound. After washing away any unbound substances, an enzyme-linked antibody is added. Following a wash, a substrate solution is added to the wells and color develops in proportion to the amount of antibody conjugate. The absorption at 450 nm is proportional to the IgG concentration.

Precautions
- Store the Rabbit IgG ELISA Assay Kit at 2-8 °C.
- The Rabbit IgG ELISA Assay Kit is for research use only. Not for use in diagnostic procedures.
- Do not use the reagents beyond the expiration date marked on box label.
- Please read the instructions carefully before using the Rabbit IgG ELISA Assay Kit.
- Do not mix reagents from different lots.
- Some components of this Rabbit IgG ELISA Assay Kit contain Thimerosal, a mercury containing compound. The stop solution contains 0.5 M sulphuric acid. Follow routine precautions for handling hazardous chemicals.

Other supplies required
- Deionized or distilled water
- Graduated cylinder
- Micropipettes, multipipette
- Microplate reader

Preparation of reagents and samples
- Bring all reagents of the Rabbit IgG ELISA Assay Kit to room temperature before use. If crystals have formed, mix gently until the crystals have completely dissolved.

- The microplate strips are ready to use. Remove excess strips (breakable) from the frame, reseal in the bag with the desiccant and store at 2-8 °C.

- Dilute the wash buffer with deionized or distilled water 1:10 (e. g. 40 ml + 360 ml water). The diluted solution is stable for 30 days at 2-8 °C.

- Use the Standard concentrate to produce a 1:2-dilution series with diluent (e. g. 250 µl + 250 µl diluent):
- Dilute the samples with diluent. If samples generate values outside the standard curve, the dilution factor may be varied. To exclude matrix effects the dilution factor should be at least 1:50.

**Assay procedure**

It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents, standard curve and samples as directed in the previous section.
2. Pipette 100 μl of samples, standards, positive control or diluent (as negative control) into the wells.
3. Seal wells with adhesive strip and incubate for 1 hour at room temperature with shaking *.
4. Aspirate fluid from wells and wash three times with 300 μl wash buffer. After the last wash, invert the plate and tap on a clean paper towel.
5. Add 100 μl of HRP conjugate to each well.
6. Seal wells with adhesive strip and incubate for 1 hour at room temperature with shaking *
7. Repeat the wash as in step 4.
8. Dispense 100 μl of TMB substrate solution into each well.
9. Incubate for 10 minutes at room temperature in the dark.
10. Add 100 μl of stop solution to each well.
11. Determine the absorbance within 30 minutes at 450 nm. A reference wavelength of 620 nm/690 nm is recommended.

*: If a MTP shaker is not available, it is possible to incubate for 2 hours without shaking.

**Calculation of results**

Create a standard curve for the Rabbit IgG ELISA Assay Kit using computer software capable of generating a curve fit (4 parameter fit; x-axis: log, IgG concentration; y-axis: linear, absorbance). As an alternative, draw a standard curve on semi-log paper (x-axis: log, IgG concentration; y-axis: linear, absorbance). The IgG concentrations can be calculated from the standard curve. The calculated concentrations must be multiplied by the sample dilution factor.
If the absorbance of some samples is outside the standard curve a subsequent determination with changed dilutions will provide a proper result.

**Typical Standard Curve:**

![Typical Standard Curve](image)

**Performance Characteristics**

- **Standard curve:**
  7 standards between 3.125 ng/ml and 200 ng/ml
- **Sensitivity:**
  156 ng/ml (sample dilution 1:50)
- **Sample materials:**
  Protein solutions, serum, body fluids
- **Intraassay precision (CV):**
  (n=10)
  - at 4.1 ng/ml: 2.9%
  - at 17.3 ng/ml: 2.5%
  - at 34.7 ng/ml: 2.6%
Materials provided:

<table>
<thead>
<tr>
<th>Number of determinations/Catalog No.</th>
<th>1x96 determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microplate strips, antibody coated</td>
<td>12 x 8</td>
</tr>
<tr>
<td>Wash buffer, 10fold conc.</td>
<td>50 ml</td>
</tr>
<tr>
<td>Diluent, ready to use</td>
<td>100 ml</td>
</tr>
<tr>
<td>Standard concentrate, 200 ng/ml ♦</td>
<td>2 ml</td>
</tr>
<tr>
<td>Anti-IgG(rabbit)-Ab., HRP conjugate, ready to use</td>
<td>12 ml</td>
</tr>
<tr>
<td>TMB substrate, ready to use</td>
<td>12 ml</td>
</tr>
<tr>
<td>Stop solution, ready to use (0.5 M sulphuric acid)</td>
<td>12 ml</td>
</tr>
</tbody>
</table>

♦: contains Thimerosal

Assay procedure summary:

A. Preparation
1. Bring all reagents to room temperature
2. Dilute wash buffer 1:10
3. Prepare the standard curve from a 1:2-dilution series of standard concentrate with diluent
4. Dilute samples with diluent

B. Performance
1. Pipette 100 μl of samples, standards, controls into the wells
2. Incubate for 1 hour at room temperature with shaking
3. Wash three times with 300 μl of wash buffer
4. Add 100 μl of HRP conjugate to each well
5. Incubate for 1 hour at room temperature with shaking
6. Wash three times with 300 μl of wash buffer
7. Dispense 100 μl of TMB substrate solution
8. Incubate for 10 minutes at room temperature in the dark
9. Add 100 μl of stop solution
10. Measure absorption at 450 nm
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*For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.*