Rabbit IgA ELISA

Catalog Number:
IGA79-K01 (1 x 96 wells)
*For Research Use Only. Not for use in diagnostic procedures.*
**Introduction**
The Eagle Biosciences Rabbit IgA ELISA Assay Kit is designed for the quantitative determination of IgA antibodies in rabbit serum and biological samples. The Rabbit IgA ELISA Assay Kit is for research use only and should not be used in diagnostic procedures.

**Principle of the Assay**
The determination of rabbit IgA is carried out as direct sandwich ELISA. An antibody specific for rabbit IgA has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IgA 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 IgA concentration.

**Precautions**
- Store the Rabbit IgA ELISA Assay Kit at 2-8 °C.
- 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 IgA ELISA Assay Kit.
- Do not mix reagents from different lots.
- Some components of this Rabbit IgA 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 IgA (Rabbit) 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 plastic bag with the desiccant and store at 2-8 °C

- Dilute the **wash buffer** with deionized or distilled water **1:10** (e. g. 50 ml + 450 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):
Rabbit IgA ELISA Assay Kit

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<table>
<thead>
<tr>
<th>Standard</th>
<th>Preparation</th>
<th>Conc. (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S 7</td>
<td>standard conc. Undiluted</td>
<td>1000</td>
</tr>
<tr>
<td>S 6</td>
<td>S 7 1:2 diluted</td>
<td>500</td>
</tr>
<tr>
<td>S 5</td>
<td>S 6 1:2 diluted</td>
<td>250</td>
</tr>
<tr>
<td>S 4</td>
<td>S 5 1:2 diluted</td>
<td>125</td>
</tr>
<tr>
<td>S 3</td>
<td>S 4 1:2 diluted</td>
<td>62.5</td>
</tr>
<tr>
<td>S 2</td>
<td>S 3 1:2 diluted</td>
<td>31.25</td>
</tr>
<tr>
<td>S 1</td>
<td>S 2 1:2 diluted</td>
<td>15.625</td>
</tr>
</tbody>
</table>

Dilute the samples with diluent. To exclude matrix effects the dilution factor should be at least 1:50. If samples generate values outside the standard curve, the dilution factor may be varied.

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, or diluent (as negative control) into the wells.
3. Seal wells with adhesive strip and incubate for 1 hour at room temperature with shaking (recommended) or 2 hours without 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 (recommended) or 2 hours without 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.
**Calculation of results**
Create a standard curve using computer software capable of generating a curve fit (4 parameter fit; x-axis: log, IgA concentration; y-axis: linear, absorbance). As an alternative, draw a standard curve on semi-log paper (x-axis: log, IgA concentration; y-axis: linear, absorbance). The IgA 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 samples dilutions will provide a proper result.

**Materials provided:**

<table>
<thead>
<tr>
<th>Number of determinations/Catalog No.</th>
<th>1x96 Determ.</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, 1000 ng/ml ♦</td>
<td>2 ml</td>
</tr>
<tr>
<td>Anti-IgA(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
5. Dilute freshly HRP conjugate 1:100 with diluent

**B. Performance**
1. Pipette 100 μl of samples, standards, diluent (blank) into the wells
2. Incubate for 1 hour at room temperature with shaking (or 2 hrs without 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 (or 2 hrs without 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.