Mouse/Rat Prolactin ELISA KIT

Cat. No.:DEIA04929
Pkg.Size:96T

Intended use

The Prolactin ELISA kit is used for the quantitative measurement of prolactin in a Mouse/ Rat plasma.

General Description

Prolactin (lactogenic hormone) is a single chain polypeptide hormone with a molecular weight of approximately 23,000 daltons. Prolactin is secreted from the anterior pituitary gland. Plasma and pituitary PRL levels are significantly greater in adult female than in male rats. This difference is thought to be brought about by ovarian steroids. A similar sex difference was found in pituitary PRL content in normal mice from 30 days of age. During and following pregnancy, prolactin, in association with other hormones, stimulates breast development and milk production. Hypersecretion of prolactin can be caused by pituitary tumors, hypothalamic diseases, hypothyroidm, renal failure, acute exercise and several medications.

Principle Of The Test

The prolactin kit is based on a solid phase sandwich ELISA method. The samples and diluted biotinylated anti-prolactin antibodies are added to the wells coated with polyclonal antibody to prolactin. Prolactin in samples binds to the anti-prolactin polyclonal antibody on the well and the biotinylated anti- prolactin antibody binds to prolactin. Unbound protein and biotin conjugate are washed off by wash buffer. Diluted horseradish peroxidase conjugated streptavidin is added to each wells and the streptavidin is allowed to bind to the biotin in the complex. Unbound enzyme conjugate is washed off. Upon the addition of the substrate, the intensity of color is proportional to the concentration of prolactin in the samples. A standard curve is prepared relating color intensity to the concentration of the prolactin.

Reagents And Materials Provided

1. Microwell coated with Prolactin polyclonal antibody 12x8x1
2. Prolactin Standards: 6 vials (ready to use) 0.5 ml
3. Biotinylated Antibody reagent (ready to use) 12 ml
4. Streptavidin Enzyme Conjugate: 1 bottle (ready to use) 12 ml
5. TMB Substrate: 1 bottle (ready to use) 12 ml
6. Stop Solution: 1 bottle (ready to use) 12 ml
7. 20X Wash concentrate: 1 bottle 25 ml

Materials Required But Not Supplied

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbance paper or paper towel
6. Graph paper
Storage

1. Store the kit at 2 - 8° C.
2. Keep microwells sealed in a dry bag with desiccants.
3. The reagents are stable until expiration of the kit.

Specimen Collection And Handling

1. Collect blood specimens and separate the plasma immediately.
2. Specimens may be stored refrigerated at (2 - 8 °C) for 5 days. If storage time exceeds 5 days, store frozen at (-20 °C) for up to one month.
3. Avoid multiple freeze-thaw cycles.
4. Prior to assay, frozen sera should be completely thawed and mixed well.

Reagent Preparation

Prepare 1X Wash buffer by adding the contents of the bottle (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18 - 26 °C).

Assay Steps

Prior to assay, allow reagents to stand at room temperature.
Gently mix all reagents before use.
1. Place the desired number of coated strips into the holder.
2. Pipet 50 µl of Prolactin standards, control and patient’s sera.
3. Add 100 µl of biotin conjugate to all wells. Shake the plate for 10 seconds to mix the solution.
4. Cover the plate and incubate for 60 minutes at room temperature (18 - 26° C).
5. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
6. Add 100 µl of streptavidin enzyme conjugate to all wells.
7. Cover the plate and incubate for 30 minutes at room temperature.
8. Remove liquid from all wells. Wash wells three times with 300 µl of 1X wash buffer. Blot on absorbance paper or paper towel.
9. Add 100 µl of TMB substrate to all wells.
10. Cover plate and incubate for 15 minutes at room temperature.
11. Add 50 µl of stop solution to all wells.
12. Read absorbance on ELISA Reader at 450 nm within 15 minutes after adding the stopping solution.

Calculation

The standard curve is constructed as follows:
1. Check prolactin standard values on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit. See example of the standard attached.
2. To construct the standard curve, plot the absorbance for the standards (vertical axis) versus the standard concentrations (horizontal axis) on a linear graph paper. Draw the best curve through the points.
3. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.
### Sensitivity

The sensitivity was determined by calculating the mean plus 2SD of the standard zero point tested 20 times in the same run. Sensitivity: 0.2 ng/ml.

### Linearity

Two different samples were diluted with the "0" calibrator to 1:2, 1:4 and 1:8. Prolactin values were assayed and results were corrected with the dilution factor. The results of these dilution tests are as follows:

<table>
<thead>
<tr>
<th>Serum</th>
<th>Original Value (ng/ml)</th>
<th>Percentage of Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>60</td>
<td>1:2 99 1:4 96 1:8 93</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>1:2 97 1:4 95 1:8 94</td>
</tr>
</tbody>
</table>

### Recovery

Known quantities of prolactin were added to a serum that contained a low concentration of Prolactin.

<table>
<thead>
<tr>
<th>Expected Value (ng/ml)</th>
<th>Recovered (ng/ml)</th>
<th>Percentage of Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>4.6</td>
<td>92</td>
</tr>
<tr>
<td>20</td>
<td>19.6</td>
<td>98</td>
</tr>
<tr>
<td>40</td>
<td>42</td>
<td>105</td>
</tr>
</tbody>
</table>

### Reproducibility

Intra-Assay:  
Inter-assay:
Precautions

1. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
2. The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
3. It is recommended that standards, control and serum samples be run in duplicate.
4. Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Limitations

1. The test results obtained using this kit is for research use. It is recommended that each lab establish normal range based on sample population.
2. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Analyte Gene Information

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Prl prolactin [ Mus musculus ]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Official Symbol</td>
<td>Prl</td>
</tr>
<tr>
<td>Synonyms</td>
<td>PRL; prolactin; Prl1a1; AV290867;</td>
</tr>
<tr>
<td>GeneID</td>
<td>19109</td>
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<tr>
<td>mRNA Refseq</td>
<td>NM_001163530</td>
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<tr>
<td>Protein Refseq</td>
<td>NP_001157002</td>
</tr>
<tr>
<td>Pathway</td>
<td>Cytokine-cytokine receptor interaction, organism-specific biosystem; Cytokine-cytokine receptor interaction, conserved biosystem; Jak-STAT signaling pathway, organism-specific biosystem; Jak-STAT signaling pathway, conserved biosystem; Neuroactive ligand-receptor interaction, organism-specific biosystem; Neuroactive ligand-receptor interaction, conserved biosystem; Prostaglandin Synthesis and Regulation, organism-specific biosystem;</td>
</tr>
<tr>
<td>Function</td>
<td>hormone activity; prolactin receptor binding;</td>
</tr>
</tbody>
</table>

REFERENCES