

ab285344 – Mouse/rat Prolactin ELISA Kit

For the quantitative measurement of Mouse/Rat Prolactin in serum and plasma.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab285344>

Storage and Stability

Store kit at 4°C. Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light. Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities.

Materials Supplied

| Item | Quantity | Storage Condition |
|-------------------------------------|------------------|-------------------|
| Prolactin Mab pre-coated Microplate | 96 wells | +4°C |
| Prolactin Standard | 0.5 mL (6 vials) | +4°C |
| Biotinylated Antibody Reagent | 12 mL | +4°C |
| Streptavidin Enzyme Conjugate | 12 mL | +4°C |
| 20X Wash Concentrate | 25 mL | +4°C |
| TMB Substrate | 12 mL | +4°C |
| Stop Solution | 12 mL | +4°C |

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Microplate reader capable of measuring absorbance at 450 nm
- Absorbent paper
- Adjustable pipettes and pipette tips.

Reagent Preparation

Prepare just the appropriate amounts for the assay.

Wash Concentrate: 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).

Δ Note: The rest of the reagents are ready to use.

Sample Preparation

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

Assay Protocol

Δ Note: Prior to assay, bring all reagents to room temperature. Gently mix all reagents before use. Check Prolactin standard value on each standard vial. This value might vary from lot to lot. Make sure you check the value on every kit.

1. Place the desired no. of coated strips into the holder. Replace any unused microwell strips back into the aluminium bag, seal and store at 2-8°C.
2. Pipette 50 µL of Prolactin Standards, control, and samples into designated wells.

3. Add 100 µL of biotin conjugate to all wells. Shake gently for 10 sec. to mix.
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 absorbent paper towels.
6. Add 100 µL of streptavidin enzyme conjugate to all wells. Cover the plate incubate for 30 minutes. at room temperature.
7. Remove liquid from all wells & wash wells three times with 300 µL of 1X wash buffer. Blot on absorbent paper towels.
8. Add 100 µL of TMB substrate to all wells & incubate for 15 minutes. at room temperature.
9. Add 50 µL of stop solution to all wells. Shake the plate gently to mix the solution.
10. Read absorbance on ELISA Reader at 450 nm within 15 minutes. after adding the stop solution.

Calculation

Construct the standard curve; plot the absorbance for the Prolactin standards (vertical axis) versus Prolactin standard concentrations (horizontal axis). Draw the best curve through the points. Read the absorbance for controls and each unknown sample from the curve. Record the value for each control or unknown sample.

Performance Characteristics

Intra-assay precision

| Samples | No. of Replicates | Mean ng/mL | Standard Deviation | Coefficient of Variation (%) |
|---------|-------------------|------------|--------------------|------------------------------|
| 1 | 16 | 30.1 | 2.1 | 6.9 |
| 2 | 16 | 16.2 | 0.8 | 4.9 |
| 3 | 16 | 5.1 | 0.3 | 5.9 |

Inter-assay precision

| Samples | No. of Replicates | Mean ng/mL | Standard Deviation | Coefficient of Variation (%) |
|---------|-------------------|------------|--------------------|------------------------------|
| 1 | 10 | 32.2 | 2.1 | 6.5 |
| 2 | 10 | 16.7 | 0.88 | 5.3 |
| 3 | 10 | 5.2 | 0.4 | 7.7 |

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

| Samples | No. of Replicates | Mean ng/mL | Standard Deviation | Mean + 2SD (Sensitivity) |
|---------------|-------------------|------------|--------------------|--------------------------|
| Zero Standard | 20 | 0.10 | 0.05 | 0.2 ng/mL |

Recovery: Known quantities of prolactin were added to sample that contained low concentration of Prolactin.

| Expected Value (ng/mL) | Recovered (ng/mL) | % of Recovery |
|------------------------|-------------------|---------------|
| 5 | 4.6 | 92 |
| 20 | 19.6 | 98 |
| 40 | 42 | 105 |

Linearity: Two different patient 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:

| Expected Value (ng/mL) | Recovered (ng/mL) | % of Recovery | | |
|------------------------|-------------------|---------------|-----|-----|
| | | 1:2 | 1:4 | 1:8 |
| 1 | 60 | 99 | 96 | 93 |
| 2 | 50 | 97 | 95 | 94 |

Warning & Precautions:

- Do not pipette by mouth.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- It is recommended that standards, control and serum samples be run in duplicate.
- 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.

Technical Support

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