EliKine™ Human Prolactin ELISA Kit

Cat #: KTE6904 Size: 48 T/96 T

	Human Prolactin ELISA Kit		
REF	**Cat #:** KTE6904	LOT	**Lot #:** Refer to product label
	Detection range: 40 μIU/mL-2000 μIU/mL		**Sensitivity:** 40 μIU/mL
	Precision: Intra-assay Precision: The CV (%) < 15%. Inter-assay Precision :The CV (%) < 15%		**Recovery:** The recovery ranged from 90% to 110% with an overall mean recovery of 100%.
	Specificity: EliKine™ Human Prolactin ELISA Kit has high sensitivity and excellent specificity for detection of Human Prolactin. No significant cross-reactivity or interference between Human Prolactin and analogues was observed.		
	Applicable samples: Serum, Plasma		
	Storage: Stored at 4℃ for 12 months, protected from light		

Assay Principle

Human prolactin (PRL) is a single-chain polypeptide hormone composed of 198 amino acids produced by the pituitary gland with a molecular weight of 22,000. It is structurally and biologically similar to growth hormone (GH) and placental prolactin (HPL). PRL acts directly on somatic cells to promote mammary gland growth, development and emulsion formation. The levels of PRL in non-pregnant women and men were comparable, and the levels of PRL in plasma increased gradually after pregnancy, peaked before delivery, and further increased during lactation. PRL is secreted in a pulsatile manner, and emotional fluctuations, trauma, etc. have a significant impact on the secretion of PRL. EliKine™ Human Prolactin ELISA Kit employs a two-site sandwich ELISA to quantitate Human Prolactin in samples. An antibody specific for Human Prolactin has been pre-coated onto a microplate. Standards and samples are added to the appropriate microtiter plate wells with HRP conjugated antibody specific for Human Prolactin and any Human Prolactin present is bound by the immobilized antibody. Following a wash to remove any unbound enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Human Prolactin in the sample. The color development is stopped and the intensity of the color is measured.

Materials Supplied and Storage Conditions

Kit components	Size		Storage conditions
	48 T	96 T	
Human Prolactin Standard (lyophilized)	1×6	1×6	4℃
HRP Conjugated Human Prolactin Detect Antibody	3 mL	6 mL	4℃
HRP Substrate A	3.5 mL	7 mL	4℃, protected from light

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HRP Substrate B	3.5 mL	7 mL	4℃, protected from light
Stop Solution	3.5 mL	7 mL	4℃
Wash Buffer (20×)	7.5 mL	15 mL	4℃
Human Prolactin Microplate	48 wells	96 wells	4℃
Plate Covers	1	2	RT

Note: Std_1: 0 μIU/mL; Std_2: 40 μIU/mL; Std_3: 100 μIU/mL; Std_4: 300 μIU/mL; Std_5: 800 μIU/mL; Std_6: 2000 μIU/mL.

Materials Required but Not Supplied

- Microplate reader capable of measuring absorbance at 450 nm
- Multi channel pipette or automated microplate washer
- Incubator, refrigerated centrifuge
- Precision pipettes, disposable pipette tips
- Deionized water

Reagent Preparation

Note: Bring all reagents equilibrate to room temperature before use. If crystals have formed in the Buffer Concentrates, warm them gently until they completely dissolved.

1×Wash buffer: Wash buffer(20×) dilute with deionized water 1:20 to obtain the 1×Wash Buffer. Store at 4℃.

Human Prolactin standard: Add 500 μL of Deionized water into the lyophilized standard vial. Dissolve the powder thoroughly by gentle shake.

Sample Preparation

1. Serum: Use a serum separator tube and allow samples to clot for 30 min at room temperature before centrifugation for 15 min at 1,000 g. Remove serum and assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.
2. Plasma: Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 min at 1,000 g within 30 min of collection. Assay immediately or aliquot and store samples at -20℃. Avoid repeated freeze-thaw cycles.

Note: Do not use grossly hemolyzed or lipemic specimens Samples should be aliquoted and must be stored at -20℃ to avoid loss of bioactive Human Prolactin. If samples are to be used within 24 hours, they may be stored at 2 to 8℃. Avoid repeated freeze-thaw cycles. Prior to assay, the frozen sample should be brought to room temperature slowly and mixed gently.

Assay Procedure

1. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
2. Add 50 μL of Human Prolactin Standard or Sample per well. It is recommended that all Standards and Samples be added in duplicate to the microplate. Set a Blank well without any solution.
3. Add 50 μL of HRP Conjugated Human Prolactin Detect Antibody to each well (not to Blank well). Mix well, cover with the plate cover provided and then incubate for 1 h at 37℃.
4. Remove liquid in each well and wash, repeating the process for a total of three washes. Wash by filling each well with 1×Wash Buffer (250 μL) using a Multi channel pipette or automated microplate washer, and let it stand for 10 s, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining 1×Wash Buffer by invert the plate and blot it against clean paper towels.
5. Add 50 μL of Substrate A and 50 μL of Substrate B to each well, mix well and cover with the plate cover provided. Incubate for 15 min at 37℃. Keeping the plate away from drafts and other temperature fluctuations in the dark.
6. Add 50 μL of Stop solution to each well. Stop Solution should be added to the plate in the same order as HRP substrate. The color in the wells should change from blue to yellow. If the color in the wells is green or if the color change does not appear

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uniform, gently tap the plate to ensure thorough mixing.

7. Determine the optical density of each well within 30 min, using a microplate reader set to 450 nm.

Data Analysis

1. Average the duplicate readings for each standard and subtract the average zero standard (Std_1) optical density (O.D.).

2. Drawing of standard curve: With the standard solution concentration as the x-axis and the mean optical density (O.D.) for each standard as the y-axis, draw the standard curve. A computer software can be used to create a standard curve.

Typical Data

Typical standard curve （$R^2 \geqslant 0.99$）

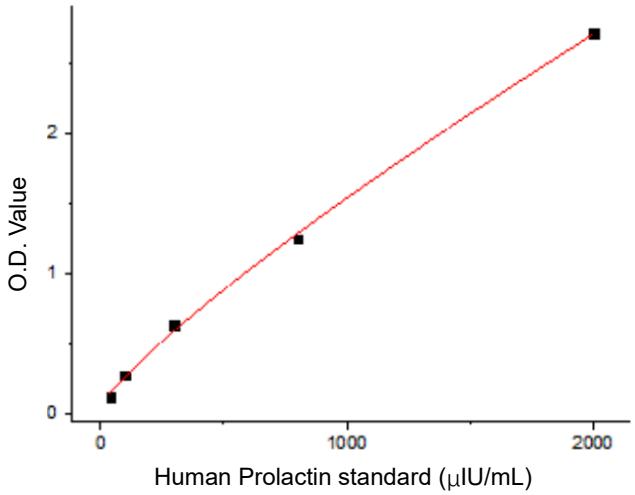

Figure1. Standard Curve of Human Prolactin in 96-well plate assay, data provided for demonstration purposes only. A new standard Curve must be generated for each assay.

Precautions

1. Do not mix or substitute reagents with those from other lots or sources.

2. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.

3. To ensure accurate results, proper adhesion of plate covers during incubation steps is necessary.

4. Stop Solution has certain Corrosive. Please take protective measures when operating.

Recommended Products

Catalog No.	Product Name
KTE6902	EliKine™ Human LH ELISA Kit
KTE6903	EliKine™ Human FSH ELISA Kit
KTE6905	EliKine™ Human TSH ELISA Kit

Disclaimer

The reagent is only used in the field of scientific research, not suitable for clinical diagnosis or other purposes.

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