Datasheet for ABIN365639

Luteinizing Hormone ELISA Kit

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Overview

Quantity:	96 tests
Target:	Luteinizing Hormone (LH)
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	2-75 mIU/mL
Minimum Detection Limit:	2 mIU/mL
Application:	ELISA

Product Details

Purpose:	For the quantitative determination of human luteinizing hormone(LH? concentrations in serum, plasma, tissue homogenates.
Sample Type:	Serum, Plasma, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of human LH.
Cross-Reactivity (Details):	Limited by current skills and knowledge, it is impossible for us to complete the cross-reactivity detection between the target antigen and all analogues for other species. Therefore, cross reaction may still exist.
Sensitivity:	0.5 mIU/mL
Components:	• Assay plate (12 × 8 coated Microwells)

Product Details

	• Standard (freeze dried) • Biotin-antibody (100 × concentrate) • HRP-avidin (100 × concentrate) • Biotin-antibody Diluent • HRP-avidin Diluent • Sample Diluent • Wash Buffer (25 × concentrate) • TMB Substrate • Stop Solution • Adhesive Strip (for 96 wells) • Instruction manual
Material not included:	• Microplate reader capable of measuring absorbance at 450nm, with the correction wavelength set at 540nm or 570nm. • An incubator which can provide stable incubation conditions up to 37°C ± 0.5°C. • Squirt bottle, manifold dispenser or automated microplate washer. • Absorbent paper for blotting the microtiter plate. • 100mL and 500mL graduated cylinders. • Deionized or distilled water. • Pipettes and pipette tips. • Test tubes for dilution.

Target Details

Target:	Luteinizing Hormone (LH)
Abstract:	LH Products
Target Type:	Hormone

Application Details

Application Notes:	• The supplier is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance. • Samples to be used within 5 days may be stored at 2-8°C, otherwise samples must be stored at -20°C (≤ 1 month) or -80°C (≤ 2 months) to avoid loss of bioactivity and contamination. • Grossly hemolyzed samples are not suitable for use in this assay. • If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary. • Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

	• Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts of certain chemicals. • Owing to the possibility of mismatching between antigens from another resource and antibodies used in this supplier's kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by this supplier's products. • Influenced by factors including cell viability, cell number and cell sampling time, samples from cell culture supernatant may not be recognized by the kit. • Fresh samples without long time storage are recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.
Comment:	Detection wavelength: 450 nm Information on standard material: Depending on the antigen to be detected, standards can be either native or recombinant protein. The recombinant proteins are being expressed in CHO cells in most cases. Please inquire for more information. The formulation of auxiliary material in the standard is considered proprietary information, however it does not contain any poisonous substance. Proclin 300 (1:3000) is used as preservative. Information on reagents: In most cases the stop solution provided is 1 N H2SO4. The formulation of wash solution is proprietary information. None of the components contain (sodium) azide, thimerosal, 2-mercaptoethanol (2-ME) or any other poisonous materials. For the sandwich method kits, the sample diluent, antibody diluent, enzyme diluent and standard all contain BSA. Information on antibodies: The antibodies provided in different kits vary in regards to clonality and host. Some antibodies are affinity purified, some are Protein A
Sample Volume:	100 µL
Assay Time:	1 - 4.5 h
Plate:	Pre-coated
Protocol:	This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for LH has been pre-coated onto a microplate. Standards and samples are pipetted into the wells with a Horseradish Peroxidase (HRP) conjugated antibody specific for LH. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color

	develops in proportion to the amount of LH bound in the initial step. The color development is stopped and the intensity of the color is measured.
Reagent Preparation:	• **Biotin-antibody (1×)** - Centrifuge the vial before opening. Biotin-antibody requires a 100-fold dilution. The suggested dilution is 10µL of Biotin-antibody + 990µL of Biotin-antibody Diluent. • **HRP-avidin (1×)** - Centrifuge the vial before opening. HRP-avidin requires a 100-fold dilution. The suggested dilution is 10µL of HRP-avidin + 990µL of HRP-avidin Diluent. • **Wash Buffer (1×)** - If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500mL of Wash Buffer (1×). • **Standard** - Centrifuge the standard vial at 6000-10000rpm for 30s. Reconstitute the Standard with 1ml of Sample Diluent. Do not substitute other diluents. This reconstitution produces a stock solution of 200pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions. Pipette 250µL of Sample Diluent into each tube. Use the stock solution to produce a 2-fold dilution series. Mix each tube thoroughly before the next transfer. The undiluted Standard serves as the high standard (200pg/mL). Sample Diluent serves as the zero standard (0ng/mL). Note: • Kindly use graduated containers to prepare the reagent. Please don't prepare the reagent directly in the Diluent vials provided in the kit. • Bring all reagents to room temperature (18-25°C) before use for 30 min. • Prepare fresh standard for each assay. Use within 4 hours and discard after use. • Making serial dilution in the wells directly is not permitted. • Please carefully reconstitute Standards according to the instruction. Avoid foaming and mix gently until the crystals have completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10µL when pipetting. • It is recommended to use distilled water to prepare reagents and samples. Using contaminated water or container for reagent preparation will influence detection result.
Sample Collection:	• **Serum**: Use a serum separator tube (SST) and allow samples to clot for two hours at room temperature or overnight at 4 °C before centrifugation for 15 minutes at 1000 × g. Remove serum and assay immediately or aliquot and store samples at -20 °C or -80 °C. Avoid repeated freeze-thaw cycles. • **Plasma**: Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 15 minutes at 1000 × g at 2-8 °C within 30 minutes of collection. Assay immediately or aliquot and store samples at -20 °C or -80 °C. Avoid repeated freeze-thaw cycles. • **Tissue Homogenates**: Rinse 100 mg tissue with 1× PBS, homogenize in 1mL of 1× PBS and store overnight at -20 °C. After two freeze-thaw cycles to break the cell membranes,

centrifuge the homogenates for 5 minutes at 5000 × g, 2-8 °C. Remove and assay the supernate immediately. Alternatively, aliquot and store samples at -20 °C or -80 °C. Centrifuge the sample again after thawing before the assay. Avoid repeated freeze-thaw cycles.

Assay Procedure:

- 1. Prepare all reagents, working standards and samples as directed in the respective sections.
- 2. Refer to the Assay Layout Sheet to determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 4°C.
- 3. Add 100µL of standard or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at 37°C. A plate layout is provided to record standards and samples assayed.
- 4. Remove the liquid of each well, don't wash.
- 5. Add 100µL of Biotin-antibody (1×) to each well. Cover with a new adhesive strip. Incubate for 1 hour at 37°C. (Biotin-antibody (1×) may appear cloudy. Warm up to room temperature and mix gently until solution appears uniform.)
- 6. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (200µL) using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher and let it stand for 2 minutes, complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
- 7. Add 100µL of HRP-avidin (1×) to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 1 hour at 37°C.
- 8. Repeat the aspiration/wash process for five times as in step 6.
- 9. Add 90µL of TMB Substrate to each well. Incubate for 20 minutes at 37°C. Protect from light.
- 10. Add 50µL of Stop Solution to each well, gently tap the plate to ensure thorough mixing.
- 11. Determine the optical density of each well within 5 minutes using a microplate reader set to 450nm. If wavelength correction is available, set to 540nm or 570nm. Subtract readings at 540nm or 570nm from the readings at 450nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450nm without correction may be higher and less accurate.

Note:

- The experiment's final results will be closely related to validity of the products, operation skills of the end users and the environmental conditions.
- Samples or reagents addition: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exced 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between

	reagent additions. Also, use separate reservoirs for each reagent. • Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed. • Washing: The wash procedure is critical. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 30 second soak period following the addition of wash buffer and/or rotating the plate 180 degrees between wash steps may improve assay precision. • Controlling of reaction time: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), TMB Substrate should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading. • TMB Substrate is easily contaminated. TMB Substrate should remain colorless or light blue until added to the plate. Please protect it from light. • Stop Solution should be added to the plate in the same order as the TMB Substrate. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the TMB Substrate.
Calculation of Results:	Average the duplicate readings for each standard and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the x-axis against the concentration on the y-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the target antigen concentration versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
Assay Precision:	Intra-assay precision (precision within an assay): Three samples of known concentration were tested twenty times on one plate to assess precision. Inter-assay precision (precision between assays): Three samples of known concentration were tested in twenty assays to assess precision. • Intra-assay: CV% less than 8%

Application Details

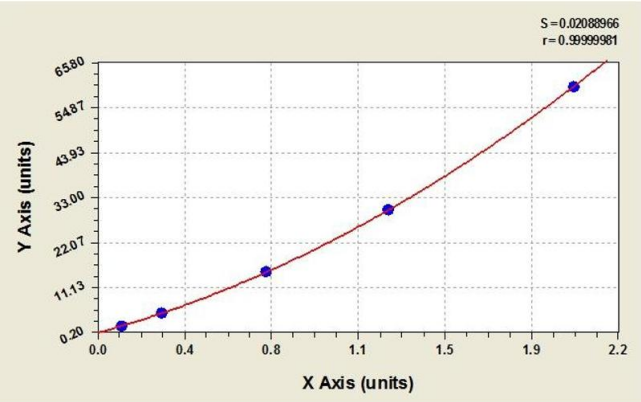
	• Inter-assay: CV% less than 10%
Restrictions:	For Research Use only

Handling

Precaution of Use:	The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face and clothing protection when using this material.
Handling Advice:	• The kit should not be used beyond the expiration date on the kit label. • Do not mix or substitute reagents with those from other lots or sources. • If samples generate values higher than the highest standard, dilute the samples with Sample Diluent and repeat the assay. • Any variation in Sample Diluent, operator, pipetting technique, washing technique, incubation time/temperature and kit age can cause variation in binding. • This assay is designed to eliminate interference by soluble receptors, binding proteins and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.
Storage:	4 °C/-20 °C
Storage Comment:	For unopened kit: All the reagents should be kept according to the labels on vials.
Expiry Date:	6 months

Publications

Product cited in:	Zheng, Liang, Wang, Zhou, Sun, Zhou: "Chronic Estradiol Administration During the Early Stage of Alzheimer's Disease Pathology Rescues Adult Hippocampal Neurogenesis and Ameliorates Cognitive Deficits in Aβ1-42 Mice." in: **Molecular neurobiology**, Vol. 54, Issue 10, pp. 7656-7669, (2018) (PubMed). Han, Wang, Liu, Li, Liu, Shen, Xu, Zhao, Zhu, Yin: "Influence of three lighting regimes during ten weeks growth phase on laying performance, plasma levels- and tissue specific gene expression- of reproductive hormones in Pengxian yellow pullets." in: **PLoS ONE**, Vol. 12, Issue 5 , pp. e0177358, (2017) (PubMed).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1.5
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Image 1.

Successfully validated (ELISA (ELISA))

by Affina Biotechnologies, Inc

Report Number: 029854

Date: Apr 10 2016

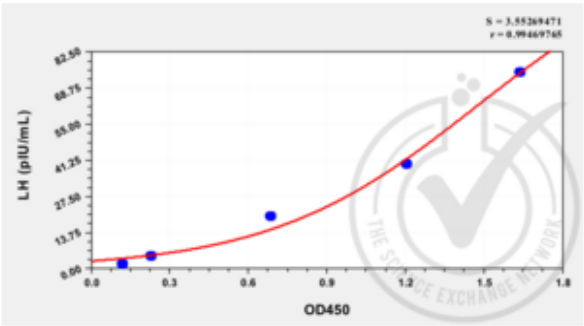
Target:	Human luteinizing hormone (LH)
Lot Number:	C4621151803
Method validated:	ELISA (ELISA)
Positive Control:	Human postmenopausal individual serum (Biochemed, Lot#BC033016HSPMG)
Negative Control:	Chicken serum (Biochemed, Lot#BC03316CSPMG)
Notes:	Human luteinizing hormone (LH) was readily detected in the positive controls (up to 4-fold dilutions). Lower dilutions of the positive control gave lower reading of LH. Spike showed fairly poor recovery (~58%) indicating presence of inhibitory components in serum (chicken) even when it was 4-fold diluted, which is consistent with the lower readings of LH in lower dilutions of human serum.
Controls:	• Positive control: Human postmenopausal female serum (Biochemed, Lot#BC033016HSPMG) • Negative control: Chicken serum (Biochemed, Lot#BC03316CSPMG) • Standard curve: 0, 2, 5, 20, 40, 75 pIU/mL LH provided in the ELISA kit • Spike control: 75 pIU/mL standard premixed with 4-fold diluted chicken serum in a 1:1 ratio. Lower dilutions of chicken serum produced lower readings.
Protocol:	• 1. 50 µL of standard and samples were added to 96-well strip plates provided in the kit with 50 µL of HRP conjugate. All samples and standards were assayed in duplicate. • 2. The microplate was covered and incubated at 37°C for 1 hr. • 3. Content of the wells was discarded and wells were washed 3 times with 200 µl of washing solution. • 4. 50 µl of Substrate A and Substrate B each was added to each well. The plate was covered and incubated at 37°C for 15 min. • 5. 50 µl of the Stop Solution was added per well. • 6. The optical density (OD value) of each well was read immediately using a microplate reader set to 450 nm. • 7. The duplicate readings for each sample were averaged and the average zero standard optical density subtracted. The corrected average-value was tabulated as Average Absorbance. A standard curve was generated by plotting the mean optical density (OD) value

for each standard on the X-axis against the concentration on the Y-axis using CurveExpert 1.4 (CUSABIO). A logistic equation was used for the best fit through the points on the graph.

- 8. The CurveExpert Analyze feature was used to calculate human LH concentrations of the samples based on their Average Absorbance values.

Experimental Notes: - The concentration of human LH in human and chicken sera was measured according to the manufacturer's directions.

Images for Validation report #029854



Validation image no. 1 for Luteinizing Hormone (LH) ELISA Kit (ABIN365639)

Figure 1: Graph of corrected-average absorbance (OD 450 nm) readings plotted for standard curve samples.

Type	Sample pIU/mL	Reading-1	Reading-2	Avg Reading	Avg Absorbance	SD	Calculated Conc
Standard Curve	75	1.708	1.717	1.712	1.674	0.006643	75
	40	1.260	1.283	1.271	1.233	0.015765	42
	20	0.710	0.765	0.738	0.700	0.039154	15
	5	0.281	0.259	0.270	0.232	0.015057	5
	2	0.165	0.156	0.160	0.122	0.006347	4
	0	0.039	0.037	0.038	0.000	0.001497	3
Spike Control	37.5	0.918	0.917	0.917	0.879	0.000419	22
Positive Control	Human serum	1.535	1.557	1.546	1.508	0.015995	62
	Human serum (2-fold dilution)	1.207	1.268	1.237	1.199	0.043212	80
	Human serum (4-fold dilution)	0.910	0.966	0.938	0.900	0.039789	92
Negative control	Chicken serum (4-fold dilution)	0.047	0.051	0.049	0.011	0.002188	12

Validation image no. 2 for Luteinizing Hormone (LH) ELISA Kit (ABIN365639)

Figure 2: Table of absorbance readings (OD 450 nm) for standard curve, spike controls, negative (chicken serum) and positive (human serum). Value for Average Reading was derived from the average of two readings (OD 450nm). The Average Reading for blank sample (no conjugate added) was subtracted from all Average Readings to yield Average Absorbance values for Standards, spike controls and control samples. Standard deviation was included for all samples. The concentration of samples was calculated using the Analyze feature of the CurveExpert 1.4 software for a logistic equation fit (Logistic Model: y=a/(1+b*exp(-cx)), a =1.2E+2, b =4.1E+1, c =2.5).