Datasheet for ABIN612677

Corticosterone ELISA Kit

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Overview

Quantity:	96 tests
Target:	Corticosterone (CORT)
Reactivity:	Human
Method Type:	Competition ELISA
Minimum Detection Limit:	90 pg/mL
Application:	ELISA

Product Details

Purpose:	The AssayMax Corticosterone ELISA kit employs a quantitative competitive enzyme immunoassay technique that measures Corticosterone in less than 3 hours
Brand:	AssayMax
Sample Type:	Plasma
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Components:	Corticosterone Microplate: A 96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody against Corticosterone. Sealing Tapes: Each kit contains 3 pre-cut, pressure-sensitive sealing tapes, which can be cut to fit the format of the individual assay. Corticosterone Standard: Corticosterone in a buffered protein base (400 ng/ml, 0.6 ml). Biotinylated Corticosterone: 1 vial, lyophilized. EIA Diluent Concentrate (10x): A 10-fold concentrated buffered protein base (20 ml). Wash Buffer Concentrate (20x): A 20-fold concentrated buffered surfactant (30 ml). 1 Streptavidin-Peroxidase Conjugate (SP Conjugate,

Product Details

	100x): A 100-fold concentrate (80µl). Chromogen Substrate: A ready-to-use stabilized peroxidase chromogen substrate tetramethylbenzidine (8 ml). Stop Solution: A 0.5 N hydrochloric acid to stop the chromogen substrate reaction (12 ml).
Material not included:	Microplate reader capable of measuring absorbance at 450 nm. Pipettes (1-20 µL, 20-200 µL, 200-1000µLand multiple channel). Deionized or distilled reagent grade water

Target Details

Target:	Corticosterone (CORT)
Alternative Name:	Corticosterone (CORT Products)
Target Type:	Hormone
Background:	Corticosterone is the adrenal steroid, the major glucocorticoid. Glucocorticoid hormones are also known as corticosteroid hormones and are synthesized mainly in the adrenal cortex, however, more recently the enzymes involved in their synthesis have been found in a variety of cells and tissues, including the heart. The effects of these hormones are mediated via both cytoplasmic mineralocorticoid receptors (MRs) and glucocorticoid receptors (GRs), which act as ligand-inducible transcription factor. Corticosterone has profound effect on the structure and function of the hippocampus. Brain corticosterone action through the glucocorticoid receptor may involve memory sotrage. Emotional stress might cause increases in plasma corticosterone.

Application Details

Sample Volume:	25 µL
Assay Time:	< 3 h
Plate:	Pre-coated
Protocol:	A polyclonal antibody specific for Corticosterone has been pre-coated onto a 96-well microplate with removable strips. Corticosterone in standards and samples is competed by a biotinylated Corticosterone sandwiched by the immobilized antibody and streptavidin-peroxidase conjugate. All unbound material is then washed away and a peroxidase enzyme substrate is added. The color development is stopped and the intensity of the color is measured.
Reagent Preparation:	Freshly dilute all reagents and bring all reagents to room temperature before use. If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved. EIA Diluent Concentrate (10x): Dilute the EIA Diluent 1:10 with reagent grade water. Store for up to 1

	month at 2-8°C. Standard Curve: Allow the standard to warm to room temperature prior to making dilutions. Prepare duplicate or triplicate standard points by serially diluting the standard solution (400 ng/ml) 4-fold with 75% volume of EIA Diluent to produce 100, 25, 6.25, 1.563, 0.391, and 0.098 ng/ml solutions. EIA Diluent serves as the zero standard (0 ng/ml). Any remaining solution should be frozen at -20°C. Standard Point Dilution [CORT] (ng/ml) P1 1 part stock (400 ng/ml) + 3 part EIA Diluent 100.000 P2 1 part P1 + 3 part EIA Diluent 25.000 P3 1 part P2 + 3 part EIA Diluent 6.250 P4 1 part P3 + 3 part EIA Diluent 1.563 P5 1 part P4 + 3 part EIA Diluent 0.391 P6 1 part P5 + 3 part EIA Diluent 0.098 P7 EIA Diluent 0.000 Biotinylated Corticosterone (2x): Dilute Biotinylated Corticosterone with 4 ml EIA Diluent to produce a 2-fold stock solution. Allow the biotin to sit for 10 minutes with gentle agitation prior to making dilutions. The stock solution should be further diluted 1:2 with EIA Diluent. Any remaining solution should be frozen at -20°C. Wash Buffer Concentrate (20x): Dilute the Wash Buffer Concentrate 1:20 with reagent grade water. SP Conjugate (100x): Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with EIA Diluent. Any remaining solution should be frozen at -20°C.
Sample Collection:	Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 2000 x g for 10 minutes and assay. Dilute human plasma 1:10, rat plasma 1:200, and mouse plasma 1:200 into EIA Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. (EDTA or Heparin can also be used as anticoagulant.) Serum: Samples should be collected into a serum separator tube. After clot formation, centrifuge samples at 2000 x g for 10 minutes. Remove serum and assay. Dilute human serum 1:10, rat serum 1:200, and mouse serum 1:200 into EIA Diluent. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Cell Culture Supernatants: Centrifuge cell culture media at 2000 x g for 10 minutes to remove debris. Collect supernatants and assay. The undiluted samples can be stored at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles. Urine: Collect urine using sample pot. Centrifuge samples at 800 x g for 10 minutes and assay. Dilute human urine 1:10, rat urine 1:20, and mouse urine 1:20 into EIA Diluent. Store samples at -20°C or below for up to 3 months. Avoid repeated freeze-thaw cycles.
Assay Procedure:	Prepare all reagents, working standards and samples as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature (20 - 30 °C). Remove excess microplate strips from the plate frame and return them immediately to the foil pouch with desiccant inside. Reseal the pouch securely to minimize exposure to water vapor and store in a vacuum desiccator. Add 25 µL of standard and/or sample per well, and immediately add 25 µL of Biotinylated Corticosterone to each well (on top of the standard or sample). Cover wells

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	with a sealing tape and incubate for two hours at room temperature. Start the timer after the last sample addition. Wash five times with 200 µL of Wash Buffer manually. Invert the plate each time and decant the contents, hit it 4-5 times on absorbent paper towel to completely remove the liquid. If using a machine wash six times with 300 µL of Wash Buffer and then invert the plate, decant the contents, hit it 4-5 times on absorbent paper towel to completely remove the liquid. Add 50 µL of Streptavidin-Peroxidase Conjugate to each well and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance. Wash the microplate as described above. Add 50 µL of Chromogen Substrate per well and incubate for about 20 minutes or until the optimal blue color density develops. Gently tap plate to ensure thorough mixing and break the bubbles in the well with pipette tip. Add 50 µL of Stop Solution to each well. The color will change from blue to yellow. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note that some unstable black particles may be generated at high concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.
Calculation of Results:	Calculate the mean value of the duplicate or triplicate readings for each standard and sample. To generate a Standard Curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance on the y-axis. The best-fit line can be determined by regression analysis using log-log or four-parameter logistic curve-fit. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor. Standard Curve The curve is provided for illustration only. A standard curve should be generated each time the assay is performed.
Assay Precision:	Intra-assay and inter-assay coefficients of variation were 4.9 % and 7.4 % respectively.
Restrictions:	For Research Use only

Handling

Handling Advice:	The kit should not be used beyond the expiration date.
Storage:	4 °C/-20 °C
Storage Comment:	Store kit at 2-8°C or -20°C upon arrival up to the expiration date. Opened EIA Diluent may be stored for up to 1 month at 2-8°C. Store reconstituted reagents at -20°C or below. Opened, unused strip wells may be returned to the foil pouch with the desiccant packets, reseal along zip-seal. May be stored for up to 1 month in a vacuum desiccator.

Publications

Product cited in:

Guillou, Romanò, Steyn, Abitbol, Le Tissier, Bonnefont, Chen, Mollard, Martin: "Assessment of lactotroph axis functionality in mice: longitudinal monitoring of PRL secretion by ultrasensitive-ELISA." in: **Endocrinology**, Vol. 156, Issue 5, pp. 1924-30, (2015) (PubMed).

Jochems, Teegarden, Chen, Boulden, Challis, Ben-Dor, Kim, Berton: "Enhancement of stress resilience through histone deacetylase 6-mediated regulation of glucocorticoid receptor chaperone dynamics." in: **Biological psychiatry**, Vol. 77, Issue 4, pp. 345-55, (2015) (PubMed).

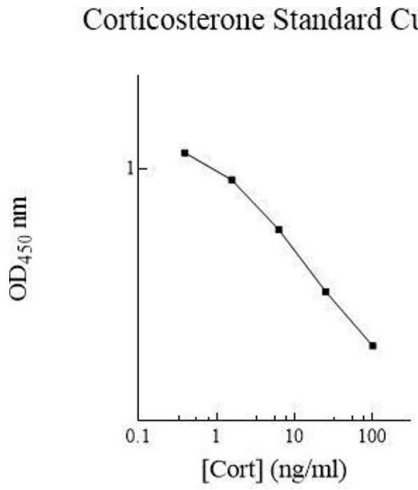
Mejia-Carmona, Gosselink, Pérez-Ishiwara, Martínez-Martínez: "Oxidant/antioxidant effects of chronic exposure to predator odor in prefrontal cortex, amygdala, and hypothalamus." in: **Molecular and cellular biochemistry**, Vol. 406, Issue 1-2, pp. 121-9, (2015) (PubMed).

Zhang, Zhao, Wang: "Chronic corticosterone exposure reduces hippocampal astrocyte structural plasticity and induces hippocampal atrophy in mice." in: **Neuroscience letters**, Vol. 592, pp. 76-81, (2015) (PubMed).

Wu, He, Ma, Wang, Ping, Wang: "Increased DNA methylation of scavenger receptor class B type I contributes to inhibitory effects of prenatal caffeine ingestion on cholesterol uptake and steroidogenesis in fetal adrenals." in: **Toxicology and applied pharmacology**, Vol. 285, Issue 2, pp. 89-97, (2015) (PubMed).

There are more publications referencing this product on: Product page

Images



ELISA

Image 1.