Datasheet for ABIN454833

CRH ELISA Kit

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Overview

Quantity:	96 tests
Target:	CRH
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	7.8-500 pg/mL
Minimum Detection Limit:	7.8 pg/mL
Application:	ELISA

Product Details

Purpose:	This immunoassay kit allows for the in vitro quantitative determination of Human corticotropin releasing hormone,CRH, concentrations in serum, tissue homogenates and other biological fluids.
Sample Type:	Serum, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay recognizes recombinant and natural human CRH .
Cross-Reactivity (Details):	No significant cross-reactivity or interference was observed.
Sensitivity:	< 3.2 pg/mL The sensitivity of this assay, or Lower Limit of Detection (LLD) was defined as the lowest detectable concentration that could be differentiated from zero.

Product Details

Characteristics:	Homo sapiens,Human,Corticoliberin,Corticotropin-releasing factor,CRF,Corticotropin-releasing hormone,CRH
Components:	Reagent (Quantity): Assay plate (1×20ml), Standard (2), Sample Diluent (1×20ml), Assay Diluent A (1×10ml), Assay Diluent B (1×10ml), Detection Reagent A (1×120 µl), Detection Reagent B (1×120 µl), Wash Buffer(25 x concentrate) (1×30ml), Substrate (1×10ml), Stop Solution (1×10ml), Plate sealer for 96 wells (5), Instruction (1)
Material not included:	Luminometer. Pipettes and pipette tips. EP tube 3 Deionized or distilled water.

Target Details

Target:	CRH
Alternative Name:	CRH (CRH Products)
Background:	CRHicotropin releasing factor (CRF, also CRH) was initially isolated from ovine hypothalamus by Vale et al., in 1981, and identified as a novel neuropeptide comprising 41 amino acid residues with molecular weight 4758. Later human CRF and rat CRF were also isolated and identified. The mouse CRF peptide is identical at amino acid level to the rat and human CRF peptides. CRF in anterior pituitary promotes the synthesis and secretion of ACTH, a main factor of hypothalamus-pituitary-adrenal (HPA) axis. In the rat and human, CRF distributes mainly in hypothalamus, but it was also found in spinal cord, stomach, spleen, duodenum, adrenal and placenta. In addition, immunochemical evidence supported the wide distribution of the peptide throughout the central nervous system (CNS) such as olfactory bulb, retina and central auditory system in the rat. In mouse brain extracts, the highest concentrations of CRF-like immunoreactivity (CRF-LI) has been detected in the median eminence and hypothalamus and also existing in the amygdala, thalamus, frontal CRHex, medulla/pons and cerebellum by radioimmunoassay. However because of the wide distribution, it is still disputing about CRF whether its blood level can reflect only the function of HPA axis. The relationships between CRF and stress, CRF and Alzheimer disease (AD) were attracted much attention recently. In fact the peptide was also suggested to regulate endocrine, autonomic and behavioral responses to stress, based on an experiment with acute and chronic stress rat models that showed endocrine function changes similar to those seen in patients with depression CRF in serial cerebrospinal fluid (CSF) of patients with depression was strikingly reduced as compared to those of normal subjects. The mean CRF and ACTH levels in the CSF of AD patients were significantly lower than those of healthy controls. Only in the CRHices of those with mild dementia, CRF was reduced significantly, thus CRF was proposed to serve as a potential neurochemical marker of early dementia and possible early AD. A large proportion of the CRF in

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	human brain was shown to be in the form of complex with its binding protein (CRF-BP). CRF molecule in the complex is unavailable for activation of 2 the CRF receptor. Accordingly reductions in total CRF do not necessarily predict reductions of bioactive free CRF, and the levels of total CRF and CRF in the form of complex (CRF/CRF-BP) were suggested to be the main factors determining the quantity of bioactive free CRF in human brain. In AD there have been observed dramatic reductions in the content of free CRF in the brain and thus displacement of CRF from CRF-BP was proposed as a possible treatment for AD. In primary neuron culture, CRF exhibited protective effect against cell death induced by amyloid-beta peptide, suggesting that disturbances in HPA axis function can occur independently of alteration in CRF mRNA levels in AD brain and further suggesting an additional role for CRF in protecting neurons against cell death. On the other hand, Yanaihara et al., demonstrated immunoreative CRF in various neuroendocrine tumors, and suggested that the blood level of the peptide might be used as a tumor marker.
Pathways:	Positive Regulation of Peptide Hormone Secretion, Hormone Activity, Negative Regulation of Hormone Secretion, cAMP Metabolic Process, Myometrial Relaxation and Contraction, Feeding Behaviour

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Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	The microtiter plate provided in this kit has been pre-coated with an antibody specific to CRH. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for CRH and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB substrate solution is added to each well. Only those wells that contain CRH, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450 nm ± 2 nm. The concentration of CRH in the samples is then determined by comparing the O.D. of the samples to the standard curve.
Reagent Preparation:	Bring all reagents to room temperature before use. Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 30 mL of Wash Buffer Concentrate into deionized or distilled water to prepare 750 mL of Wash Buffer. Standard - Reconstitute the Standard with 1.0 mL of Sample Diluent.

	This reconstitution produces a stock solution of 500 pg/ml. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making serial dilutions (Making serial dilution in the wells directly is not permitted). The undiluted standard serves as the high standard (500 pg/ml). The Sample Diluent serves as the zero standard (0 pg/ml). 4 pg/mL 500 250 125 62.5 31.2 15.6 7.8 0 Detection Reagent A and B - Dilute to the working concentration using Assay Diluent A and B (1:100), respectively.
Sample Collection:	Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at approximately 1000 × g. Remove serum and assay immediately or aliquot and store samples at -20 C or -80 C . Tissue homogenates - The preparation of tissue homogenates will vary depending upon tissue type. For this assay, tissue was rinsed with 1X PBS to remove excess blood, homogenized in 20 mL of 1X PBS and stored overnight at ≤ -20 C After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g. Remove the supernate and assay immediately or aliquot and store at ≤ -20 C . Other biological fluids - Remove particulates by centrifugation and assay immediately or aliquot and store samples at -20 C or -80 C . Avoid repeated freeze-thaw cycles. Note: Serum and tissue homogenates to be used within 7 days may be stored at 2-8 C , otherwise samples must stored at -20 C (≤ 1 months) or -80 C (≤ 2 months) to avoid loss of bioactivity and contamination. Avoid freeze-thaw cycles. When performing the assay slowly bring samples to room temperature.
Assay Procedure:	Allow all reagents to reach room temperature (Please do not dissolve the reagents at 37 C directly.). All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. Keep appropriate numbers of strips for 1 experiment and remove extra strips from microtiter plate. Removed strips should be resealed and stored at 4 C until the kits expiry date. Prepare all reagents, working standards and samples as directed in the previous sections. 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. 1. Add 100 µ l of Standard, Blank, or Sample per well. Cover with the Plate sealer. Incubate for 2 hours at 37 C . 2. Remove the liquid of each well, don ' t wash. 3. Add 100 µ l of Detection Reagent A working solution to each well. Cover with the Plate sealer. Incubate for 1 hour at 37 C . Detection Reagent A working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform. 4. Aspirate each well and wash, repeating the process three times for a total of three washes. Wash by filling each well with Wash Buffer (approximately 400 µ l) using a squirt bottle, multi-

	channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to 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. 5. Add 100 µ l of Detection Reagent B working solution to each well. Cover with a new Plate sealer. Incubate for 1 hours at 37 C . 6. Repeat the aspiration/wash as in step 4. 7. Add 90 µ l of Substrate Solution to each well. Cover with a new Plate sealer. Incubate within 30 minutes at 37 C . Protect from light. 8. Add 50 µ l of Stop Solution to each well. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. 9. Determine the optical density of each well at once, using a microplate reader set to 450 nm. 5 Important Note: 1. Absorbance is a function of the incubation time. Therefore, prior to starting the assay it is recommended that all reagents should be freshly prepared prior to use and all required strip-wells are secured in the microtiter frame. This will ensure equal elapsed time for each pipetting step, without interruption. 2. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals have completely dissolved. The reconstituted Standards can be used only once. This assay requires pipetting of small volumes. To minimize imprecision caused by pipetting, ensure that pipettors are calibrated. It is recommended to suck more than 10 µ l for once pipetting. 3. 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. 4. For each step in the procedure, total dispensing time for addition of reagents to the assay plate should not exceed 10 minutes. 5. 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. 6. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. 7. Duplication of all standards and specimens, although not required, is recommended. 8. Substrate Solution is easily contaminated. Please protect it from light.
Calculation of Results:	Average the duplicate readings for each standard, control, and sample and subtract the average

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	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 6 points on the graph. The data may be linearized by plotting the log of the N-MID-OT concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. It is recommended to use some related software to do this calculation, such as curve expert 13.0. 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.
Restrictions:	For Research Use only

Handling

Handling Advice:	1. The kit should not be used beyond the expiration date on the kit label. 2. Do not mix or substitute reagents with those from other lots or sources. 3. If samples generate values higher than the highest standard, further dilute the samples with the Assay Diluent and repeat the assay. Any variation in standard diluent, operator, pipetting technique, washing technique,incubation time or temperature, and kit age can cause variation in binding. 4. This assay is designed to eliminate interference by soluble receptors, ligands, 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:	The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20 °C upon being received. The other reagents can be stored at 4 °C.