Datasheet for ABIN956169

Urocortin 2 ELISA Kit

2 Images

Go to Product page

Overview

Quantity:	96 tests
Target:	Urocortin 2 (UCN2)
Reactivity:	Rat
Method Type:	Competition ELISA
Application:	ELISA

Product Details

Purpose:	The Rat Urocortin 2 ELISA is for the quantitative determination of urocortin 2 in rat plasma and serum.
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Characteristics:	This ELISA kit for determination of rat urocortin 2 in samples is based on a competitive enzyme immunoassay using a combination of highly specific antibody to rat urocortin 2 and biotin-avidin affinity system. Calibrator or samples, and labeled antigen are added to the wells of the plate coated with rabbit anti-rat urocortin 2 antibody for competitive immunoreaction. After incubation and plate washing, horseradish peroxidase (HRP) labeled streptoavidin (SA) is added to form HRP labeled streptoavidin-biotinylated antigen-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by 3,3',5,5'-Tetramethylbenzidine (TMB) and the concentration of rat urocortin 2 is calculated.
Components:	1. Antibody-Coated Plate Microtiter plate: 1 plate (96-well) Rabbit anti-rat urocortin 2 antibody 2. Urocortin 2 Calibrator Lyophilized: 1 vial (100 ng) Synthetic rat urocortin 2 3. Labeled Antigen Lyophilized 1 vial Biotinylated rat urocortin 2

Product Details

	4. SA-HRP Solution Liquid: 1 bottle (12 mL) HRP-labeled streptoavidin 5. Enzyme Substrate Liquid: 1 bottle (12 mL) TMB (3,3',5,5'-tetramethylbenzidine) Solution 6. Stop Solution Liquid: 1 bottle (12 mL) 1 M H2SO4 7. Buffer Solution Liquid: 1 bottle (26 mL) Citrate buffer 8. Wash Solution Concentrate Liquid: 1 bottle (60 mL) Concentrated saline 9. Plate Seal: 3 sheets.
Material not included:	Photometer for microtiter plate (plate reader), which can read absorbance up to 2.6 at 460 nm Washing device for microtiter plate and dispenser with aspiration system Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips Glass test tubes for preparation of calibrator solution Graduated cylinder (1,000 mL) Distilled water or de-ionized water

Target Details

Target:	Urocortin 2 (UCN2)
Alternative Name:	Urocortin 2 (UCN2 Products)
Background:	Urocortin 2 (Ucn 2), also known as stresscopin-related peptide, is a novel predicted neuropeptide related to corticotropin- releasing factor (CRF). The peptide consisting of 38 amino acid residues was first demonstrated to be expressed centrally and to bind selectively to type 2 CRF receptor (CRFR2). In rodents, Ucn 2 transcripts were shown to be expressed in the discrete regions of the central nervous system including stress-related cell groups in the hypothalamus and brainstem. More recently, the expression of Ucn 2 transcripts was detected in the olfactory bulb, pituitary, cortex, hypothalamus, and spinal cord. Ucn 2 mRNA was also found to be expressed widely in a variety of peripheral tissues, most highly in the skin and skeletal muscle tissues. Ucn 2-like immunoreactivity was detected by RIA in acid extracts of mouse brain, muscle, and skin. Immunohistochemically Ucn 2 was found in both skin epidermis and adnexal structures and in the skeletal muscle myocytes. Ucn 2 gene transcription was stimulated in the hypothalamus and brainstem by glucocorticoid administration to the mouse and inhibited by removal of glucocorticoids by adrenalectomy, suggesting a putative link between the CRFR1 and CRFR2 pathways. On the other hand, in the rat a stressor-specific regulation of Ucn 2 mRNA expression in the hypothalamic paraventricular nucleus was demonstrated, which raised the possibility of a modulary role of Ucn 2 mRNA in stress-induced alteration of anterior and posterior pituitary function, depending on the type of stress. Administration of dexamethasone to the mouse resulted in a decrease of Ucn 2 mRNA levels in

the back skin region. Adrenalectomy significantly increased Ucn 2 mRNA levels in the skin, and the levels were reduced back to normal levels after corticoid replacement.
CRFR2 is found in cardiomyocytes and in endothelial and smooth muscle cells of the systemic vasculature. Ucn 2 is expressed in the mouse cardiomyocytes. In the mouse, Ucn 2 treatment augmented heart rate, exhibited potent inotropic and lusitropic actions on the left ventricle, and induced a downward shift of the diastolic pressure-volume relation. Ucn 2 also reduced systemic arterial pressure, associated with a lowering of systemic arterial elastance and systemic vascular resistance. The effects of Ucn 2 were specific to CRFR2 function and independent of beta-adrenergic receptors. These experiments demonstrated the potent cardiovascular physiologic actions of Ucn 2 in both wild-type and cardiomyopathic mice and support a potential beneficial use of Ucn 2 in congestive heart failure treatment. The use of Ucn 2 was also proposed to treat ischemic heart disease because of its potent cardioprotective effect in the mouse heart and its minimal impact on the hypothalamic stress axis. Administration of Ucn 2 to the mouse prevented the loss of skeletal muscle mass resulting from disuse due to casting, corticosteroid treatment, and nerve damage. In addition, Ucn 2 treatment prevented the loss of skeletal muscle force and myocyte cross-sectional area that accompanied muscle mass losses resulting from disuse due to casting. In normal muscles of the mouse, Ucn 2 increased skeletal muscle mass and force. It was thus proposed that Ucn 2 might find utility in the treatment of skeletal muscle wasting diseases including age-related muscle loss or sarcopenia. Mouse urocortin 2 (Ucn 2) is a new peptide predicted from mouse cDNA sequence and its physiologic and pathophysiologic significance has not yet been fully elucidated. However, the experimental data presented to date provided evidence for the important physiologic roles of Ucn 2 and urge the necessity of further investigation of the peptide from various points of view. We have already developed a mouse urocortin 2 (Ucn2) ELISA kit (KT-376) and a mouse urocortin 3 (Ucn3) 3 ELISA (KT- 377). This time, as a part of tools for urocortin research, our laboratory developed rat urocortin 2 (Ucn2) ELISA kit (KT- 493), which is highly specific for rat Ucn 2 with almost no cross-reaction to Ucn 1 (mouse, rat), Ucn 2 (mouse), Ucn 3 (mouse), and CRF (mouse, rat, human). The kit can be used for measurement of Ucn 2 in rat plasma or serum with high sensitivity. It will be a specifically useful tool for Ucn 2 research.This ELISA kit is used for quantitative determination of urocortin 2 in rat plasma & serum samples. The kit is characterized by its sensitive quantification and high specificity. In addition, it is not influenced by other components in samples. The rat urocortin 2 calibrator is a highly purified synthetic product. The ELISA kit has high specificity to rat urocortin 2 and shows no cross-reactivity to urocortin 1 (mouse, rat), urocortin 2 (mouse), urocortin 3 (mouse), nor CRF (mouse, rat, human).

Target Details

Pathways:	Positive Regulation of Peptide Hormone Secretion, Negative Regulation of Hormone Secretion, cAMP Metabolic Process

Application Details

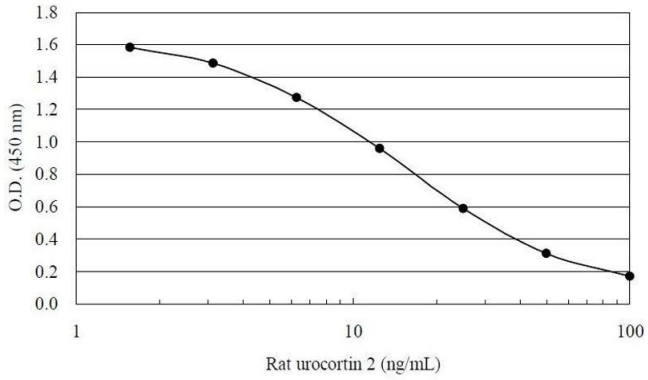
Plate:	Pre-coated
Reagent Preparation:	1. Preparation of Calibrator Solution: Reconstitute the Rat Urocortin 2 Calibrator with 1 mL of buffer solution, which makes a 100 ng/mL Calibrator Solution. Add 0.2 mL of the 100 ng/mL Calibrator Solution with 0.2 mL of Buffer Solution, which yields a 60 ng/mL Calibrator Solution. Repeat the serial dilution procedure to make 26, 12.6, 6.26, 3.126 and 1.663 ng/mL Calibrator Solutions. Buffer Solution is used as the 0 ng/mL Calibrator. Note: Calibrator Solution should be prepared immediately before use. 2. Preparation of labeled antigen: Reconstitute labeled antigen with 6 mL of distilled water. Note: Labeled Antigen Solution should be prepared immediately before use. 3. Preparation of Wash Solution: Dilute 60 mL of Wash Solution Concentrate to 1,000 mL with distilled or de-ionized water. Note: During storage of Wash Solution Concentrate at 4°C, precipitates may be observed. However, they will be dissolved when diluted. Diluted Wash Solution is stable for 6 months at 4°C. 4. The other reagents are ready for use.
Assay Procedure:	1. Bring all the reagents and samples to room temperature (20-30°C) before starting assay. 2. Add 0.3 mL/well of diluted Wash Solution into the wells of the plate, and then aspirate the solution. Repeat this washing procedure twice (total 3 times). Finally, invert the plate and tap onto an absorbent surface, such as paper toweling, to ensure removal of most of the residual Wash Solution. 3. Add 60 µL of Buffer Solution, and then add 60 µL of each prepared Calibrator Solution (0, 1.663, 3.126, 6.26, 12.6, 26, 60 and 100 ng/mL) or samples and finally add 60 µL of labeled antigen into the wells. The total pipetting time of calibrator solutions and samples for a whole plate should not exceed 30 min. 4. Cover the plate with a Plate Seal and incubate at 4°C overnight for 16 18 hours. (Still, plate shaker not needed.) 5. After incubation, move the plate back to room temperature for about 40 minutes and remove the Plate Seal, aspirate the solution in the wells and wash the wells 4 times with approximately 0.3 mL/well of diluted Wash Solution. Finally, invert the plate and tap onto an absorbent surface, such as paper toweling, to ensure removal of most of the residual Wash Solution. 6. Pipette 100 µL of SA-HRP Solution into each of the wells. 7. Cover the plate with a Plate Seal and incubate at room temperature for 2 hours. During

Application Details

	incubation, the plate should be rotated with a plate rotator. 8. Remove the Plate Seal and aspirate the solution in the wells and then wash the wells 4 times with approximately 0.3 mL/well of diluted Washing Solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure removal of most of the residual Washing Solution. 9. Add 100 µL of TMB solution into each of the wells, cover the plate with a Plate Seal and incubate for 30 minutes at room temperature, protected from light. (Still, plate shaker not needed.) 10. Add 100 µL of Stop Solution into each of the wells. 11. Read optical absorbance of the solution in the wells at 460 nm. Read plate optical absorbance of reaction solution in wells as soon as possible after stopping the color reaction. Notes: Perform all determinations in duplicate.
Calculation of Results:	The dose-response curve of this assay fits best to a 4 (or 6)-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4 (or 6)-parameter logistic function. Otherwise calculate mean absorbance values of wells containing calibrators and plot a calibration curve on semilogarithmic graph paper (abscissa: concentration of calibrator, ordinate: absorbance values). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from this calibration curve. When a sample value exceeds 100 ng/mL, it needs to be diluted with buffer solution to a proper concentration.
Restrictions:	For Research Use only

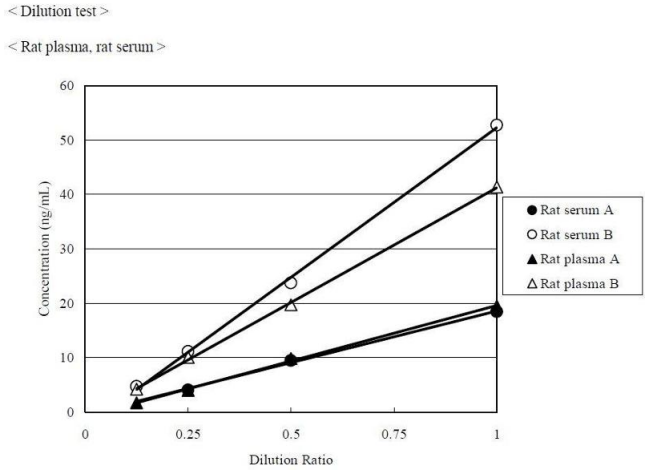
Handling

Storage:	4 °C



ELISA

Image 1.



ELISA

Image 2.

