KAMIYA BIOMEDICAL COMPANY

Mouse Urocortin 2 EIA

For the quantitative determination of Urocortin 2 in mouse plasma and serum.

Cat. No. KT-376

For Research Use Only.

Rev. 15693376

PRODUCT INFORMATION

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INTENDED USE
The Mouse Urocortin 2 EIA is for the quantitative determination of Urocortin 2 in mouse plasma and serum. For research use only.

INTRODUCTION
Urocortin 2 (Ucn 2), also known as Stresscopin-Related Peptide, is a novel predicted neuropeptide related to corticotropin-releasing factor (CRF). The peptide consists of 38 amino acid residues and 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, especially in the skin and skeletal muscle tissues. Ucn 2-like immunoreactivity was detected by RIA in acid extracts of mouse brain, muscle, and skin. Ucn 2 was found by immunohistochemical staining 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 adrenal-ectomy, suggesting a putative link between the CRFR1 and CRFR2 pathways. On the other hand, in rats 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 mice 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 mice, 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 the 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 cardio-protective effect in the mouse heart and its minimal impact on the hypothalamic stress axis.

Administration of Ucn 2 to mice 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. Advantages of this kit include high sensitivity, high specificity, no interference with other sample components. The mouse Urocortin 2 Calibrator is a highly purified synthetic product.

PRINCIPLE
This EIA kit for the determination of mouse Urocortin 2 is based on a competitive enzyme immunoassay using a combination of a highly specific antibody to mouse Urocortin 2 and a biotin-avidin affinity system. The 96-well plate is coated with rabbit anti-mouse Urocortin 2 antibody. Mouse Urocortin 2 calibrator or samples and labeled antigen are added to the wells for a competitive immuno-reaction. After incubation and washing, HRP-labeled streptoavidin (SA-HRP) is added to form HRP-labeled streptoavidin-biotinylated mouse Urocortin 2-antibody complexes on the surface of the wells. Finally, HRP enzyme activity is determined by o-Phenylenediamine dihydrochloride (OPD), and the concentration of mouse Urocortin 2 is calculated.

COMPONENTS

Component	Form	Quantity	Main Ingredient
1. Antibody-Coated Plate	MTP*1	1 plate (96-well)	Rabbit anti-mouse Urocortin 2 antibody
2. Urocortin 2 Calibrator	Lyophilized	1 vial (200 ng)	Synthetic mouse Urocortin 2
3. Labeled Antigen	Lyophilized	1 vial	Biotinylated mouse Urocortin 2
4. SA-HRP Solution	Liquid	1 bottle (12 mL)	HRP-labeled streptoavidin
5. Substrate Buffer	Liquid	1 bottle (24 mL)	0.015% Hydrogen Peroxide
6. OPD Tablet	Tablet	2 tablets	o-Phenylenediamine dihydrochloride
7. Stop Solution	Liquid	1 bottle (12 mL)	1 M H_2SO_4
8. Buffer Solution	Liquid	1 bottle (15 mL)	Citrate buffer
9. Wash Solution Concentrate	Liquid	1 bottle (50 mL)	Concentrated saline
10. Plate Seal		3 sheets	

MTP*1 ..... Microtiter plate

MATERIALS REQUIRED BUT NOT PROVIDED

- Photometer for microtiter plate (plate reader), which can read absorbance up to 2.5 at 490 or 492 nm
- Rotator for microtiter plate
- 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

PRECAUTIONS

Protect reagents from strong light (e.g. direct sunlight) during storage and assay.

Satisfactory performance of the test is guaranteed only when reagents are used from a kit with identical lot number.

As pipetting operations may affect the precision of the assay, precisely pipette the prepared Calibrator Solutions or samples into corresponding wells. Use a new tip for each calibrator or sample to avoid cross-contamination. Use clean test tubes or vessels.

Always run a calibration curve when testing samples.

REAGENT PREPARATION

1. Preparation of Calibrator Solutions: Reconstitute the Urocortin 2 Calibrator (lyophilized mouse Urocortin 2, 200 ng /vial) with 1 mL of Buffer Solution, giving a 200 ng/mL Calibrator Solution after reconstitution. 0.1 mL of the reconstituted Calibrator Solution is diluted with 0.2 mL of Buffer Solution to yield a 66.7 ng/mL Calibrator Solution. Repeat the serial dilution to make Calibrator Solutions at 22.2, 7.41, 2.47, and 0.82 ng/mL. Buffer Solution is used as the zero calibrator (0 ng/mL).

   Note: Calibrator Solution must be prepared immediately before assay. Use clean test tubes or vessels.

2. Preparation of Labeled Antigen: Reconstitute Labeled Antigen with 6 mL of distilled water.

   Note: Labeled Antigen must be prepared immediately before assay. Use clean test tubes or vessels.

3. Preparation of Substrate Solution: Dissolve OPD Tablet in 11 mL of Substrate Buffer.

   Note: Substrate Solution must be prepared immediately before use. Use clean test tubes or vessels.

4. Preparation of Wash Solution: Dilute 50 mL of Wash Solution Concentrate to 1,000 mL with distilled or de-ionized water.

   Note: Diluted Wash Solution is stable for 6 months when stored at 4°C. During storage of the Wash Solution Concentrate at 4°C, precipitates may be observed, however, they will dissolve when diluted.

5. Other reagents are ready for use.

STORAGE

Store kit at 4°C. This kit can be used dividedly in strips of the plate. In such case, the rest of reconstituted reagents (calibrator and labeled antigen) should be stored at or below -30°C (stable for 1 month).

SPECIMEN COLLECTION AND HANDLING

EDTA-2Na (1 mg/mL) additive blood collection tube is recommended for the plasma collection. Plasma or serum samples must be used as soon as possible after collection. If the samples are to be tested at a later time, they should be divided into test tubes in small amounts and frozen at or below –30°C. Avoid repeated freeze/thaw cycles.

ASSAY PROTOCOL

1. Warm the reagents and samples to room temperature (20 - 30°C) before beginning the test.
2. Add 0.35 mL of diluted Wash Solution into the wells and aspirate the Washing Solution in the wells. Repeat this washing procedure twice, for a total of 3 wash steps. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
3. Add 25 µL Buffer Solution into the wells, then add 20 µL of the prepared Calibrator Solutions (0, 0.82, 2.47, 7.41, 22.22, 66.7, 200 ng/mL) or samples into wells. Add 50 µL of Labeled Antigen into the wells. The total pipetting time of calibrator solutions and samples for a whole plate should not exceed 30 minutes.
4. Cover the plate with the Plate Seal and incubate at 4°C for 16-18 hours. Plate rotator not needed for this step.
5. Incubate plate for 40 minutes at room temperature. Plate rotator not needed for this step.
6. Remove the Plate Seal and aspirate the solution in the wells. Wash the wells four times with approximately 0.35 mL/well of diluted Wash Solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
7. Pipette 100 µL of SA-HRP Solution into each of the wells.
8. Cover the plate with a Plate Seal and incubate at room temperature for 2 hours. During the incubation, the plate should be rotated on a plate rotator (~ 100 rpm).
9. Remove the Plate Seal, aspirate and wash the wells four times with approximately 0.35 mL/well of diluted Wash Solution. Finally, invert the plate and tap it onto an absorbent surface, such as paper toweling, to ensure blotting free of most residual washing solution.
10. Add 100 µL of Substrate Solution into the wells, cover the plate with a Plate Seal and incubate for 20 minutes at room temperature. Plate rotator not needed for this step.
11. Add 100 µL of Stop Solution into the wells to stop the reaction.
12. Read the optical absorbance of the wells at 490 or 492 nm. The optical absorbance of reaction solution in wells should be read as soon as possible after stopping the color reaction.

Note: Test all samples in duplicate.

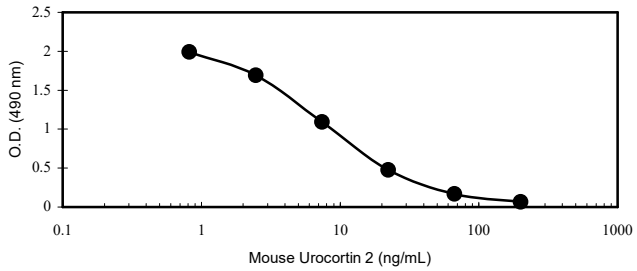
RESULTS

The dose-response curve of this assay fits best to a 4 or 5-parameter logistic equation. The results of unknown samples can be calculated with any computer program having a 4 or 5-parameter logistic function. Otherwise calculate mean absorbance values of wells containing calibrators and plot a calibration curve on semi logarithmic graph paper (abscissa: concentation of calibrator; ordinate; absorbance values). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from this calibration curve.

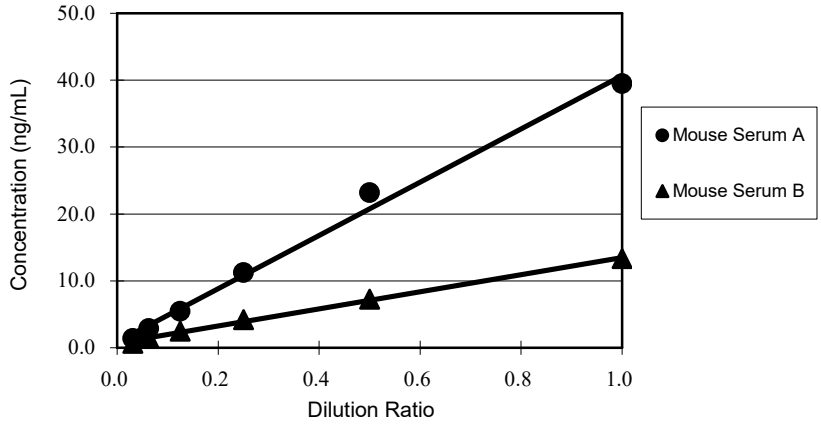
When a sample value exceeds 200 ng/mL, it must be diluted with Buffer Solution and re-assayed until the sample value is within the assay range.

PERFORMANCE

Typical Calibration Curve (example only, a new calibration curve for each run must be established by the end-user)



Dilution Test



Analytical Recovery

	Mouse Urocortin 2 Added (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
Mouse Plasma A	0.0	1.58		
	1.0	2.92	2.58	113.18
	5.0	7.36	6.58	111.85
	30.0	35.82	31.58	113.43
	50.0	59.92	51.58	116.17
Mouse Plasma B	0.0	1.72		
	1.0	2.71	2.72	99.63
	5.0	6.73	6.72	100.15
	30.0	35.99	31.72	113.46
	50.0	60.79	51.72	117.54
Mouse Plasma C	0.0	1.67		
	1.0	2.64	2.67	98.88
	5.0	7.07	6.67	106.00
	30.0	30.89	31.67	97.54
	50.0	55.80	51.67	107.99
Mouse Plasma D	0.0	1.30		
	1.0	2.62	2.30	113.91
	5.0	7.11	6.30	112.86
	30.0	32.96	31.30	105.30
	50.0	49.97	51.30	97.41
Mouse Serum A	0.0	2.69		
	1.0	4.02	3.69	108.94
	5.0	8.57	7.69	111.44
	30.0	38.24	32.69	116.98
	50.0	70.07	52.69	132.99
Mouse Serum B	0.0	2.66		
	1.0	3.91	3.66	106.83
	5.0	8.78	7.66	114.62
	30.0	44.14	32.66	135.15
	50.0	78.51	52.66	149.09

Mouse Serum C	0.0	2.96		
	1.0	4.14	3.96	104.55
	5.0	9.12	7.96	114.57
	30.0	43.45	32.96	131.83
	50.0	78.94	52.96	149.06
Mouse Serum D	0.0	2.51		
	1.0	3.59	3.51	102.28
	5.0	8.48	7.51	112.92
	30.0	38.72	32.51	119.10
	50.0	71.82	52.51	136.77

Precision and Reproducibility

- Intra-assay CV (%) Serum and Plasma 6.71 – 9.01, 2.51 – 5.25
- Inter-assay CV (%) Serum and Plasma 6.36 – 11.12, 4.70 – 8.28

Assay Range

0.82 – 200 ng/mL

Cross-Reactivity

ACTH, mouse and rat 0.61%

No cross-reactivity with mouse or rat Urocortin 1, mouse or rat Urocortin 3, mouse, rat or human CRF.

FOR RESEARCH USE ONLY

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