Mouse Urocortin 2 ELISA

Cat. No.: RSCYK190R

1. Introduction

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)[1)]. In the rodent, 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[1)]. More recently, the expression of Ucn 2 transcripts was detected in the olfactory bulb, pituitary, cortex, hypothalamus, and spinal cord[2)]. 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[3)]. Ucn 2-like immunoreactivity was detected by RIA in acid extracts of mouse brain, muscle, and skin[3)]. Immunohistochemically Ucn 2 was found in both skin epidermis and adnexal structures and in the skeletal muscle myocytes[3)]. 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[2)]. 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[4)]. Administration of dexamethasone to the mouse resulted in a decrease of Ucn 2 mRNA levels in the back skin region. Adrenalectomy signifcantly increased Ucn 2 mRNA levels in the skin, and the levels were reduced back to normal levels after corticoid replacement[3)].

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[5)]. 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[5]. 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[6].

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[7].

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 succeeded this time in the development of mouse urocortin 2 EIA kit which highly specific for mouse Ucn 2 with almost no crossreaction to Ucn 1 (mouse, rat), Ucn 3 (mouse), ACTH (mouse, rat) and CRF (mouse, rat, human). The kit can be used for measurement of Ucn 2 in mouse plasma or serum with high sensitivity. It will be a specifically useful tool for Ucn 2 research.

RSCYK190R Mouse Urocortin 2 EIA Kit

- ▼ The assay kit can measure mouse urocortin 2 in the range of 0.82 - 200 ng/mL.
- ▼ The assay completes within 16-18 hr.＋3 hr.
- ▼ With one assay kit, 41 samples can be measured in duplicate.
- ▼ Test sample: Mouse plasma & serum
  Sample volume: 20 µL
- ▼ The 96-well plate in kit was consisted by 8-wells strips. The kit can be used separately.
- ▼ Precision and reproducibility
  Intra-assay CV (%)
  Mouse plasma 2.51-5.25 Mouse serum 6.71-9.01
  Inter-assay CV (%)
  Mouse plasma 4.70-8.28 Mouse serum 6.36-11.12
- ▼ Stability and Storage
  Store all of the components at 2-8°C.
  18 months from the date of manufacturing.
  The expiry date is described on the label of kit.

Contents

1) Antibody coated plate
2) Standard
3) Labeled antigen
4) SA-HRP solution
5) Substrate buffer
6) OPD tablet
7) Stopping solution
8) Buffer solution
9) Washing solution (concentrated)
10) Adhesive foil

2. Characteristics

This EIA kit is used for quantitative determination of urocortin 2 in mouse plasma & serum samples. The kit is characterized for sensitive quantification, high specificity and no influence with other components in samples. Mouse urocortin 2 standard is highly purified synthetic product.

Specificity

The EIA kit has high specificity to mouse urocortin 2 and shows cross reactivity neither urocortin 1 (mouse, rat), urocortin 3 (mouse), ACTH (mouse, rat) nor CRF (mouse, rat, human).

Test Principle

This EIA kit for determination of mouse urocortin 2 in samples is based on a competitive enzyme immunoassay using combination of highly specific antibody to mouse urocortin 2 with biotin-avidin affinity system. The 96 wells plate is coated with rabbit anti mouse urocortin 2 antibody. Mouse urocortin 2 standard or samples, labeled antigen are added to the wells for competitive immunoreaction. After incubation and plate washing, HRP labeled streptoavidin (SA-HRP) are added to form HRP labeled streptoavidin-biotinylated mouse urocortin 2-antibody complex 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.

3. Composition

Component	Form	Quantity	Main Ingredient
1. Antibody coated plate	MTP*1	1 plate (96 wells)	Rabbit anti mouse urocortin 2 antibody
2. Standard	lyophilized	1 vial	Synthetic mouse urocortin (200 ng/vial)
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 hydrochloride
7. Stopping solution	liquid	1 bottle (12 mL)	1M H_2SO_4
8. Buffer solution	liquid	1 bottle (15 mL)	Phosphate buffer
9. Washing solution (Concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
10. Adhesive foil		3 sheets	

MTP*1……..Microtittration plate

4. Method

Equipment required

1. Photometer for microtitration plate (Plate reader), which can read extinction 2.5 at 492 nm

2. Microtiter plate shaker

3. Washing device for microtiter plate and dispenser with aspiration system

4. Micropipettes, multi-channel pipettes for 8 wells or 12 wells and their tips

5. Test tubes for preparation of standard solution

6. Graduated cylinder (1,000 mL)

7. Distilled water or deionized water

Preparatory work

1. Preparation of standard solution:
   Reconstitute the mouse urocortin 2 standard with 1 mL of buffer solution, which affords 200 ng/mL standard solution. The 0.1 mL of the reconstituted standard solution is diluted with 0.2 mL of buffer solution that yields 66.7 ng/mL standard solution. Repeat the same dilution to make each standard of 22.2, 7.41, 2.47, 0.82 ng/mL. Buffer solution is used as 0ng/mL.

2. Preparation of labeled antigen:
   Reconstitute labeled antigen with 6 mL of distilled water.

3. Preparation of substrate solution:
   Resolve OPD tablet with 11 mL of substrate buffer. It should be prepared immediately before use.

4. Preparation of washing solution:
   Dilute 50 mL of washing solution (concentrated) to 1000 mL with distilled or deionized water.

5. Other reagents are ready for use.

Procedure

1. Before beginning the test bring all the reagents and samples to room temperature (20 ~ 30°C).

2. Add 0.35 mL/well of washing solution into the wells and aspirate the washing solution in the wells. Repeat this washing procedure further twice (total 3 times).

3. Fill 25 μL of buffer solution into the wells first, then introduce 20 μL of each of standard solutions (0, 0.82, 2.47, 7.41, 22.2, 66.7, 200 ng/mL) or samples and finally add 50 μL of labeled antigen into the wells.

4. Cover the plate with adhesive foil and incubate it at 4ºC overnight for 16 ~ 18 hours. (Still, plate shaker not need)

5. After 4°C incubation, move the plate back to room temperature waiting for 40 minutes and take off the adhesive foil, aspirate and wash the wells four times with approximately 0.35 mL/well of washing solution.

6. Pipette 100 μL of SA-HRP solution into the wells.

7. Cover the plate with adhesive foil and incubate it at room temperature (20 ~ 30ºC) for 2 hour. During the incubation, the plate should be shake with a plate shaker.

8. Resolve OPD tablet with 11 mL of substrate buffer. It should be prepared immediately before use.

9. Take off the adhesive foil, aspirate and wash the wells four times with approximately 0.35 mL/well of washing solution.

10. Add 100 μL of substrate solution into the wells, cover the plate with adhesive foil and incubate it for 20 minutes at room temperature.

11. Add 100 μL of stopping solution into the wells to stop color reaction.

12. Read the optical absorbance of the wells at 492 nm. Calculate mean absorbance values of wells containing standards and plot a standard curve on semilogarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance values). Use the standard curve to read mouse urocortin 2 concentrations in samples from the corresponding absorbance values.

5. Notes

1. Samples must be used as soon as possible after collection. If the samples are tested later, they should be divided into test tubes in small amount and frozen at or below -30 ºC. Avoid repeated freezing and thawing of samples.

2. Mouse urocortin 2 standard, labeled antigen and substrate solution should be prepared immediately before use.

3. During storage of washing solution (concentrated) at 2-8 ºC, precipitates may be observed, however they will be dissolved when diluted. Diluted washing solution is stable for 6 months at 2-8 ºC.

4. Pipetting operations may affect the precision of the assay, pipette standard solutions or samples precisely into each well of plate. In addition, using clean test tubes or vessels in assay and use new tip for each standard or sample to avoid cross contamination.

5. When sample value exceeds 200 ng/mL, it needs to be diluted with buffer solution to proper concentration.

6. During incubation in the room temperature except color reaction, the test plate should be shake gently by plate shaker to promote immunoreaction.

7. Perform all the determination in duplicate.

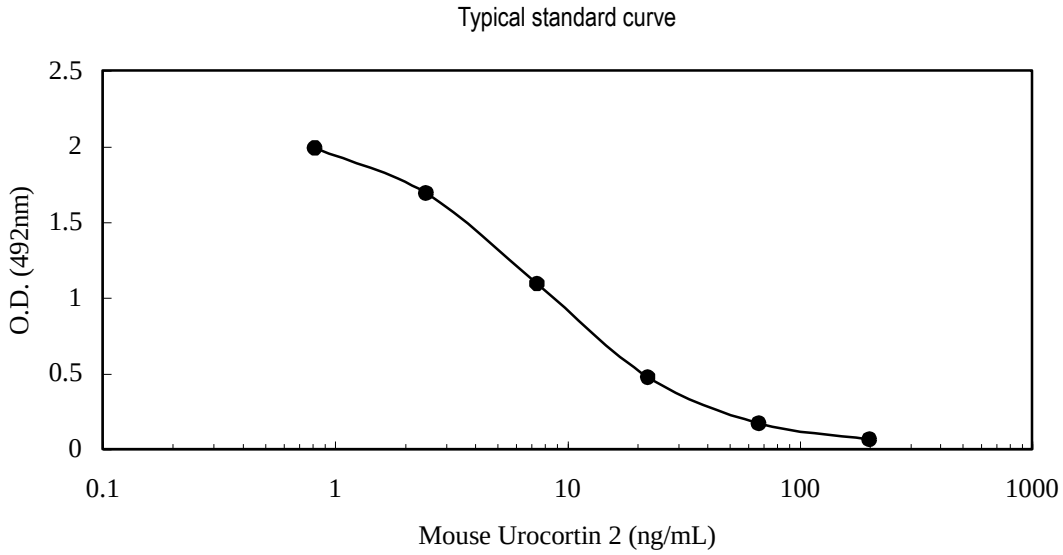
8. Read plate optical absorbance of reaction solution in wells as soon as possible after stopping color reaction.

9. To quantitate accurately, always run a standard curve when testing samples.

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

11. Satisfactory performance of the test is guaranteed only when reagents are used from combination pack with identical lot number.

6. Performance Characteristics



Analytical recovery

Mouse Plasma A

Added Urocortin 2 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
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

Added Urocortin 2 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
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

Added Urocortin 2 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
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

Added Urocortin 2 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
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

Added Urocortin 2 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
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

Added Urocortin 2 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
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

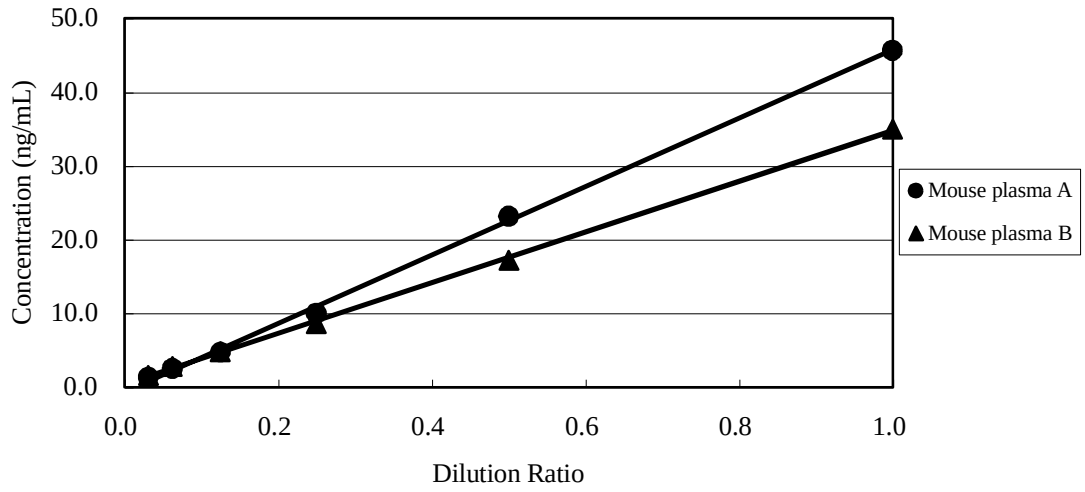
Added Urocortin 2 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	2.96		
1.0	4.14	3.96	104.55
5.0	9.12	7.96	114.57
30.0	43.45	32.96	131.83
50.0	78.94	52.96	149.06

Mouse Serum D

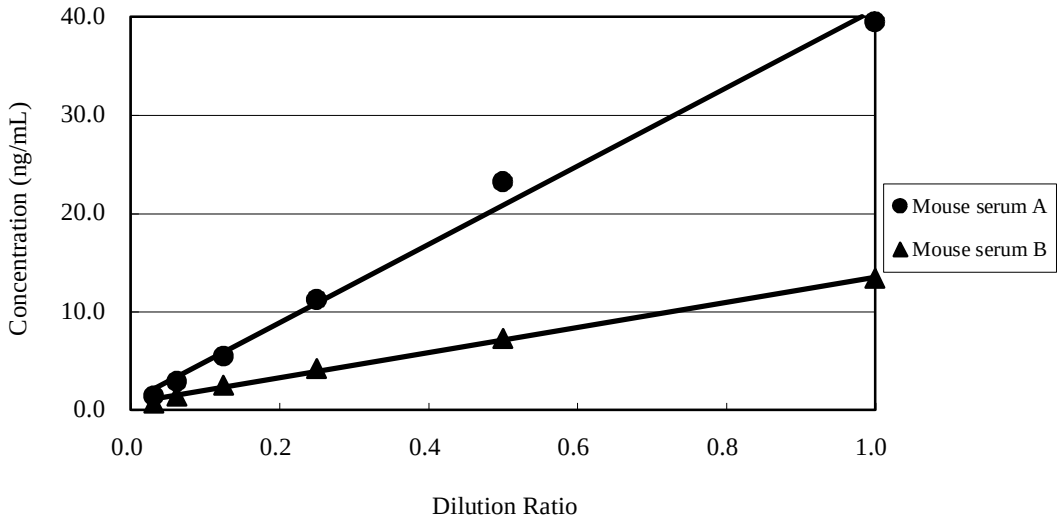
Added Urocortin 2 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	2.51		
1.0	3.59	3.51	102.28
5.0	8.48	7.51	112.92
30.0	38.72	32.51	119.10
50.0	71.82	52.51	136.77

Dilution test

Mouse plasma



Mouse serum



Crossreactivity

Related peptides	Crossreactivity
Urocortin 2 (mouse)	100
Urocortin 1 (mouse, rat)	0
Urocortin 3 (mouse, rat)	0
ACTH (mouse, rat)	0.61
CRF (mouse, rat, human)	0

Precision and reproducibility

Mouse plasma & serum

- Intra-assay CV (%) 6.71-9.01
- Inter-assay CV (%) 6.36-11.12

Assay range

0.82 – 200 ng/mL

7. Stability and Storage

Storage Store all of the components at 2-8°C.

Shelf life 6 months from the date of manufacturing

The expiry date is described on the label of kit.

Package For 96 tests per 1 kit including standards

8. References

1. Reyes TM. (2001) Urocortin II: a member of the corticotropin-releasing factor (CRF) neuropeptide family that is selectively bound by type 2 CRF receptors. *Proc Natl Acad Sci USA.* **98**, 2843-2848
2. Chen A. (2003) Glucocorticoids regulate the expression of the mouse urocortin II gene: a putative connection between the corticotropin-releasing factor receptor pathways. *Mol Endocrinol.,***17,** 1622-1639
3. Chen A. (2004) Urocortin II gene is highly expressed in mouse skin and skeletal muscle tissues: localization, basal expression in corticotropin-releasing factor receptor (CRFR) 1-and CRFR2-null mice, and regulation by glucocorticoids. *Endocrinology.,* **145,** 2445-2457
4. Tanaka Y. (2003) Effect of stress and adrenalectomy on urocortin II mRNA expression in the hypothalamic paraventricular nucleus of the rat. *Neuroendocrinology.,* **78,** 1-11
5. Bale TL. (2004) The cardiovascular physiologic actions of urocortin II: acute effects in murine heart failure. *Proc Natl acad Sci U S A.,* **101,** 3697-3702
6. Brar BK. (2004) Urocortin II and urocortin III are cardioprotective against ischemia reperfusion injury: an essential endogenous cardioprotective role for corticotropin releasing factor receptor type 2 in the murine heart. *Endocrinology*., **145,** 24-35
7. Hinkle RT. (2003) Urocortin II treatment reduces skeletal muscle mass and function loss during atrophy and increases nonatrophying skeletal muscle mass and function. *Endocrinology.,* **144,** 4939-4946

Gentaur Molecular Products
Voortstraat 49
1910 Kampenhout, Belgium
http://www.gentaur-worldwide.com