YK191 Rat Urocortin 2 EIA

FOR LABORATORY USE ONLY

YANAIHARA INSTITUTE INC.
2480 - 1 AWAKURA, FUJINOMIYA-SHI
SHIZUOKA, JAPAN 418 - 0011

Contents

- Please read all the package insert carefully before beginning the assay -

YK191 Rat Urocortin 2 EIA Kit

I. 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 modularly 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 significantly 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 have already developed mouse/rat urocortin 1 (Ucn1) EIA kit (YK210), mouse urocortin 2 (Ucn2)

EIA kit (YK190) and mouse/rat urocortin 3 (Ucn3) EIA kit (YK200). This time, as a part of tools for urocortin research, our laboratory developed rat urocortin 2 (Ucn2) EIA kit (YK191), which highly specific for rat Ucn 2 with almost no crossreaction to Ucn 1 (mouse, rat), Ucn 2 (mouse), Ucn 3 (mouse, rat), 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 rat Ucn 2 research.

YK191 Rat Urocortin 2 EIA Kit

- The assay kit can measure rat urocortin 2 within the range of 1.563-100 ng/mL.
- The assay is completed within 16-18 hr.＋3 hr.
- With one assay kit, 40 samples can be measured in duplicate.
- Test sample: Rat plasma and serum
  Sample volume: 50 μL
- The 96-wells plate in kit is consisted by 8-wells strips, and the strips can be used separately.
- Precision and reproducibility
  Intra-assay CV (%)
  Rat plasma 4.04-11.09　Rat serum　5.19-10.59
  Inter-assay CV (%)
  Rat plasma 2.35-11.44　Rat serum　4.72-7.88
- Stability and Storage
  Store all of the components at 2-8ºC.
  The kit is stable under the condition for 15 months from the date of manufacturing.
  The expiry date is stated on the label of kit.

Contents

1) Antibody coated plate
2) Standard
3) Labeled antigen
4) SA-HRP solution
5) Enzyme substrate solution (TMB)
6) Stopping solution
7) Buffer
8) Washing solution (concentrated)
9) Adhesive foil

II. Characteristics

This EIA kit is used for quantitative determination of urocortin 2 in rat plasma and serum samples. The kit is characterized by its sensitive quantification and high specificity. In addition, it has no influence by other components in samples. Rat urocortin 2 standard is highly purified synthetic product.

< Specificity >

This EIA kit has high specificity to rat urocortin 2 and shows no crossreactivity to urocortin 1 (mouse, rat), urocortin 2 (mouse), urocortin 3 (mouse, rat) and CRF (mouse, rat, human).

< Assay principle >

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

Ⅲ. Composition

	Component	Form	Quantity	Main Ingredient
1.	Antibody coated plate	microtiter plate	1 plate (96 wells)	Rabbit anti rat urocortin 2 antibody coated
2.	Standard	lyophilized	1 vial (100 ng)	Synthetic rat urocortin 2
3.	Labeled antigen	lyophilized	1 vial	Biotinylated rat urocortin 2
4.	SA-HRP solution	liquid	1 bottle (12mL)	Horseradish peroxidase labeled streptoavidin
5.	Enzyme substrate solution	liquid	1 bottle (12 mL)	3, 3',5 ,5'-Tetramethylbenzidine (TMB)
6.	Stopping solution	liquid	1 bottle (12 mL)	1M H_2SO_4
7.	Buffer	lyophilized	1 vial	Citrate buffer
8.	Washing solution (concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
9.	Adhesive foil		3 pieces	

IV. Method

< Equipment required >

1. Photometer for microtiter plate (plate reader) which can read extinction 2.5 at 450 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. Glass 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 rat urocortin 2 standard with 1 mL of buffer solution, which affords 100ng/mL standard solution. The reconstituted standard solution (0.2 mL) is diluted with 0.2 mL of buffer solution that yields 50 ng/mL standard solution. Repeat the dilution procedure to make each standard solution of 25, 12.5, 6.25, 3.125 and 1.563 ng/mL. Buffer solution itself is used as 0 ng/mL standard solution.
2. Preparation of labeled antigen:
   Reconstitute labeled antigen with 6 mL of distilled water.
3. Preparation of buffer solution:
   Reconstitute buffer with 15 mL of distilled water.
4. Preparation of washing solution:
   Dilute 50 mL of washing solution (concentrated) to 1,000 mL with distilled or deionized water.
5. Other reagents are ready for use.

< Procedure >

1. Before starting the assay, bring all the reagents and samples to room temperature (20 ~ 30ºC).

2. Fill 0.3 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). 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 50μL of buffer solution to the wells first, then introduce 50μL of each of standard solutions (0, 1.563, 3.125, 6.25, 12.5, 25, 50 and 100 ng/mL) or samples and finally add 50μL of labeled antigen to the wells. The total pipetting time of standard solutions and samples for a whole plate should not exceed 30 minutes.

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

5. After incubation, move the plate back to room temperature keeping for approximately 40 minutes (keep still, plate shaker not need) and take off the adhesive foil, aspirate and wash the wells 4 times with 0.3 mL/well of washing 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.

6. Add 100μL of SA-HRP solution to each of the wells.

7. Cover the plate with adhesive foil and incubate it at room temperature for 2 hours.
   During the incubation, the plate should be shaken with a plate shaker (approximately 100 rpm).

8. Take off the adhesive foil, aspirate and wash the wells 4 times with 0.3 mL/well of washing 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.

9. Add 100μL of Enzyme substrate solution (TMB) to each of the wells, cover the plate with adhesive foil and keep it for 30 minutes at room temperature in a dark place for color reaction (keep still, plate shaker not need).

10. Add 100μL of stopping solution to each of the wells to stop color reaction.

11. Read the optical absorbance of the solution in the wells at 450 nm. 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 standards and plot a standard curve on semi logarithmic graph paper (abscissa: concentration of standard; ordinate: absorbance

values). Use the average absorbance of each sample to determine the corresponding value by simple interpolation from this standard curve.

V. Notes

1. EDTA-2Na (1 mg/mL) additive blood collection tube is recommended for the plasma collection. Serum and plasma 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°C. Avoid repeated freezing and thawing of samples.

2. Standard, buffer and labeled antigen solutions should be prepared immediately before use. This kit can be used dividedly in strips of the plate. In such case, the rest of reconstituted reagents (standard, buffer and labeled antigen) should be stored at or below -30°C (stable for 1 month).

3. The total pipetting time of standard solutions and samples for a whole plate should not exceed 30 minutes.

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

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

6. When sample concentration exceeds 100 ng/mL, it needs to be diluted with buffer solution to proper concentration.

7. During the incubation with SA-HRP solution at room temperature, the assay plate should be shaken gently by a plate shaker to promote immunoreaction (approximately 100 rpm).

8. Perform all the determination in duplicate.

9. Read plate optical absorbance of reaction solution in wells as soon as possible after stop color reaction.

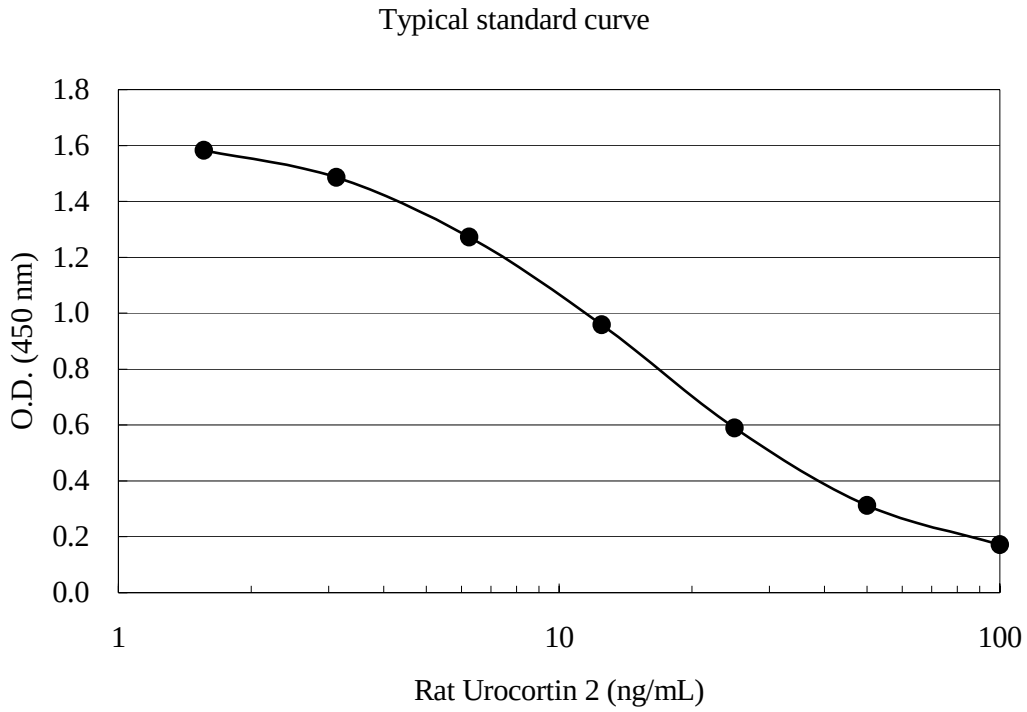
10. To quantitate accurately, always run a standard curve when testing samples.

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

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

13. Some reagents contain animal serum, care should be taken when handling.

VI. Performance Characteristics



<Analytical recovery>

<Rat serum 1>

Added Rat Urocortin 2 (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.0	1.48	-	-
10.0	13.00	11.48	113.24
20.0	20.97	21.48	97.63
40.0	40.75	41.48	98.24

<Rat serum 2>

Added Rat Urocortin 2 (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.0	3.04	-	-
10.0	12.26	13.04	94.02
20.0	20.86	23.04	90.54
40.0	46.04	43.04	106.97

<Rat serum 3>

Added Rat Urocortin 2 (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.0	3.02	-	-
10.0	13.03	13.02	100.08
20.0	21.83	23.02	94.83
40.0	40.81	43.02	94.86

<Rat serum 4>

Added Rat Urocortin 2 (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.0	2.89	-	-
10.0	12.78	12.89	99.15
20.0	20.48	22.89	89.47
40.0	41.01	42.89	95.62

<Rat plasma 1>

Added Rat Urocortin 2 (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.0	1.90	-	-
10.0	10.23	11.90	85.97
20.0	19.19	21.90	87.63
40.0	40.51	41.90	96.68

<Rat plasma 2>

Added Rat Urocortin 2 (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.0	2.01	-	-
10.0	11.48	12.01	95.59
20.0	19.37	22.01	88.01
40.0	38.04	42.01	90.55

<Rat plasma 3>

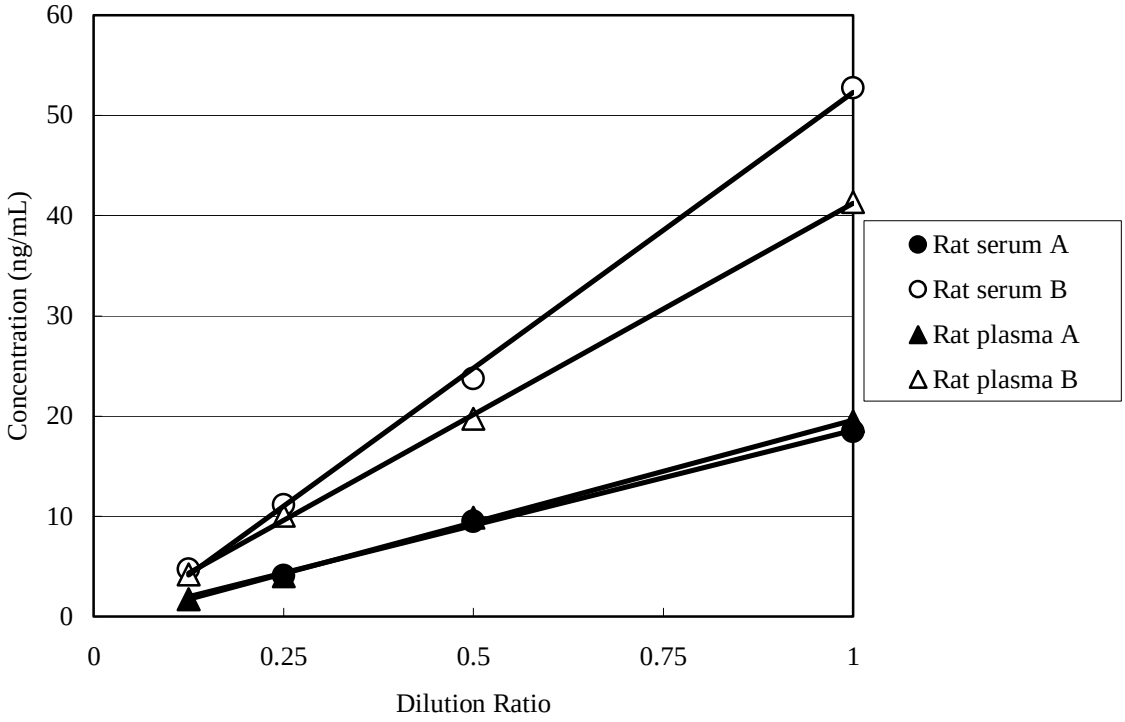
Added Rat Urocortin 2 (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.0	1.84	-	-
10.0	10.18	11.84	85.98
20.0	18.03	21.84	82.55
40.0	37.77	41.84	90.27

<Rat plasma 4>

Added Rat Urocortin 2 (ng/mL)	Observed (ng/mL)	Expected (ng/mL)	Recovery (%)
0.0	0.81	-	-
10.0	9.56	10.81	88.44
20.0	19.16	20.81	92.07
40.0	35.30	40.81	86.50

< Dilution test >

< Rat plasma, rat serum >



<Crossreactivity>

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

<Precision and reproducibility>

Test sample	Intra-assay CV(%)	Inter-assay CV(%)
Rat plasma	4.04-11.09	2.35-11.44
Rat serum	5.19-10.59	4.72-7.88

<Assay range>

1.563 ~ 100 ng/mL

VII. Stability and Storage

< Storage > Store all of the components at 2~8ºC.

< Shelf life > The kit is stable under the condition for 15 months from the date of manufacturing. The expiry date is stated on the label of kit.

< Package > For 96 tests per one kit including standards

VIII. 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

<Manufacturer>
Yanaihara Institute Inc.
2480-1 Awakura, Fujinomiya-shi
Shizuoka, Japan 418-0011
TEL: +81-544-22-2771 FAX: +81-544-22-2770
Website: http://www.yanaihara.co.jp E-mail: ask@yanaihara.co.jp

Update at Jan. 19, 2012