COSMO BIO CO., LTD.
Inspiration for Life Science

Mouse/Rat Urocortin 1 EIA

Cat. No. YII-YK210-EX

FOR LABORATORY USE ONLY

COSMO BIO CO., LTD.
Inspiration for Life Science
TOYO 2CHOME, KOTO-KU, TOKYO, 135-0016, JAPAN
http://www.cosmobio.co.jp e-mail : export@cosmobio.co.jp
Phone : +81-3-5632-9617 FAX : +81-3-5632-9618

Contents

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

YII-YK210-EX Mouse/Rat Urocortin 1 EIA Kit

Ⅰ. Introduction

Urocortin (Urocortin1: Ucn1) is first identified in rat [1], and later in human[2] and mouse[3]. It is the second mammalian member of the CRF family. Rat and mouse Ucn1 have the same amino acid sequence and display 95% structure homology to human Ucn1, 45% to CRF and 63% to urotensin.

In the rat, Ucn1 immunoreactivity (IR) was shown to distribute widely in central nervous system, endocrine organs, and digestive system and its concentration was highest in pituitary (11 pmol/g, w.w.) [4]. Koziez, Yanaihara et al. used a polyclonal antibody against rat Ucn1 to define the distribution of Ucn1-IR in rat central nervous system and found a large number of neurons with Ucn1-IR in rat brain [5].

Synthetic human Ucn1 binds with high affinity to CRF receptor type 1(CRFR1), 2 alpha(CRFR2α) and 2 beta (CRFR2β). CRFR1 and CRFR2 have been shown to link to the development of stress-related disorders, such as mood and eating disorders. CRFR1 is expressed predominantly in the brain and pituitary, whereas CRFR2 expression is limited to particular brain areas and to some peripheral organs [6]. Data were also presented supporting the hypothesis that this peptide is the endogenous ligand for the CRFR2. [5]

Synthetic human Ucn1 stimulates cAMP accumulation in cells stably transfected with those receptors and acts in vitro to release ACTH from dispersed rat anterior pituitary cells. In addition, the CRF-binding protein binds human Ucn1 with high affinity and can prevent Ucn1-stimulated ACTH secretion in vitro[2]. Ucn1 was suggested to play important roles in various tissues in normal rats, but shown not to behave as a hypothalamic hypophysiotropic factor in mediating adrenocorticotropin secretin in adrenalectomized rats [4]. Ucn1 has been implicated in various endocrine responses, such as blood pressure regulation, as well as in higher cognitive functions [5].

Synthetic human Ucn1 also stimulates plasma ACTH, cortisol and atrial natriuretic peptide (ANP) secretin and suppresses plasma ghrelin in healthy male volunteers[7]. In the human, plasma Ucn1 is elevated in heart failure, especially in its early stages. This fact may useful in the diagnosis of early heart failure [8].

We have already developed mouse urocortin 2 (Ucn2) EIA kit (YII-YK190-EX), rat urocortin 2 (Ucn2) EIA kit (YII-YK191-EX) and mouse/rat urocortin 3 (Ucn3) EIA kit (YII-YK200-EX). This time, as a part of tools for urocortin research, our laboratory developed mouse/rat Ucn1 EIA kit (YII-YK210-EX), which is highly specific for mouse/rat Ucn1 with almost no crossreaction with Ucn2 (mouse), Ucn2 (rat), Ucn3 (mouse, rat), ACTH (mouse, rat), ACTH (human) and CRF (mouse, rat, human). The kit can be used for measurement of Ucn1 in mouse/rat plasma or serum with high sensitivity. It will be a specifically useful tool for Ucn1 research.

YK210 Mouse/Rat Urocortin 1 EIA Kit	Contents
▼ This assay kit can measure mouse/rat Ucn1 in mouse/rat plasma or serum within the range of 1.563-100 ng/mL. ▼ The assay completes within 16-18 hr + 3 hr. ▼ With one assay kit, 40 samples can be measured in duplicate. ▼ Test sample: mouse/rat plasma or serum Sample volume: 10 µL ▼ The 96-wells plate of this kit consists of 12 8-wells strips, so that divided use by the strips is possible at your option. ▼ Precision and reproducibility Intra-assay CV (%): Rat serum 2.87-9.48 Rat plasma 1.70-13.01 Mouse serum 3.51-5.73 Mouse plasma 3.14-5.32 Inter-assay CV (%): Rat serum 4.44-7.76 Rat plasma 5.71-15.72 Mouse serum 5.45-9.83 Mouse plasma 8.70-10.12 ▼ Stability and storage Store all of the components at 2-8ºC. The kit is stable under the condition for 6 months from the date of manufacturing. The expiry date is indicated on the label of the kit.	1) Antibody coated plate 2) Standard 3) Labeled antigen 4) SA-HRP solution 5) Enzyme substrate solution (TMB) 6) Stopping solution 7) Buffer solution 8) Washing solution (concentrated) 9) Adhesive foil

Ⅱ. Characteristics

This EIA kit is used for quantitative determination of Ucn 1 in mouse/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. Mouse/rat Ucn1 standard is highly purified synthetic product.

< Specificity >

The EIA kit has high specificity to mouse/rat Ucn1 and shows crossreactivity with none of Ucn2 (mouse), Ucn2 (rat), Ucn3 (mouse, rat), ACTH (mouse, rat), ACTH (human) and CRF (mouse, rat, human).

< Assay principle >

This EIA kit for determination of mouse/rat Ucn1 in samples is based on a competitive enzyme immunoassay using combination of highly specific antibody to mouse/rat Ucn1 with biotin-avidin affinity system. The 96-wells plate is coated with rabbit anti-mouse/rat Ucn1 antibody. Mouse/rat Ucn1 standard or samples and biotin labeled antigen are added to the wells for competitive immunoreaction. After incubation and plate washing, horse radish peroxidase (HRP) labeled streptoavidin (SA) is added to form HRP labeled SA-biotinylated mouse/rat Ucn1-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by 3,3',5,5'-Tetramethyl benzidine (TMB) and the concentration of mouse/rat Ucn1 is calculated.

Ⅲ. Composition

Component	Form	Quantity	Main ingredient
1. Antibody coated plate	Microtiter plate	1 plate (96 wells)	Rabbit anti-mouse /rat Ucn1 antibody coated
2. Standard	lyophilized	1 vial (100 ng)	Synthetic mouse/rat Ucn1
3. Labeled antigen	lyophilized	1 vial	Biotinylated mouse/rat Ucn1
4. SA-HRP solution	liquid	1 bottle (12 mL)	Horse radish 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 solution	liquid	1 bottle (15mL)	Tris-HCl/saline buffer
8. Washing solution (concentrated)	liquid	1 bottle (50 mL)	Concentrated salineh
9. Adhesive foil		3 pieces	

Ⅳ. Method

< Equipments 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 tips
5. Test tubes for preparation of standard solution
6. Graduated cylinder (1,000 mL)
7. Distilled or deionized water

< Preparatory work >

1. Preparation of standard solution:
   Reconstitute mouse/rat Ucn1 standard antigen with 1 mL of buffer solution, which affords 100 ng/mL standard solution. The reconstituted standard solution (0.1 mL) is diluted with 0.1 mL of buffer solution, yielding 50 ng/mL standard solution. Repeat the same dilution procedure to make 25, 12.5, 6.25, 3.125 and 1.563 ng/mL standard solutions. Buffer solution itself is used as 0 ng/mL standard solution.
2. Preparation of labeled antigen solution:
   Reconstitute labeled antigen with 6 mL of distilled or deionized water.
3. Dilution of plate washing solution (concentrated):
   Dilute 50 mL of plate washing solution (concentrated) to 1,000 mL with distilled or deionized water.
4. Other reagents are ready for use.

< Procedure >

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

2. Add 0.3 mL/well of plate washing solution into each of the wells and then aspirate it. 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. Fill 40 µL of buffer solution into the wells first, then introduce 10 µL 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 solution. The total pipetting time of standard solutions and samples for a whole plate should not exceed 30 min.

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 about 40 minutes (keep still, plate shaker not need) and take off the adhesive foil, aspirate and wash the wells 4 times with approximately 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. Pipette 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 incubation, the plate should be shaken with a plate shaker.

8. Take off the adhesive foil, aspirate the solutions in the wells and then wash the wells 4 times with approximately 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 well, cover the plate with 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 enzyme reaction stopping solution into each of the wells.

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 semilogarithmic 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. It is strongly recommended that 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 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 and labeled antigen) should be stored at -30°C.

3. During storage of plate washing solution (concentrated) at 2~8ºC, recipitates may be observed. However they will be dissolved when diluted.

4. Pipetting operations may affect precision of the assay, so that pipette standard solutions or samples precisely into each well of assay plate. Test tubes and vessels used in assay must be clean and new tips must be used for each standard solution or sample to avoid cross contamination.

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

6. Except color reaction with TMB, the assay plate should be shaken gently by a plate shaker to promote immunoreaction during incubation at room temperature.

7. Perform all assays in duplicate.

8. After stop enzyme reaction, read optical absorbance of reaction solutions in the wells of assay plate as soon as possible.

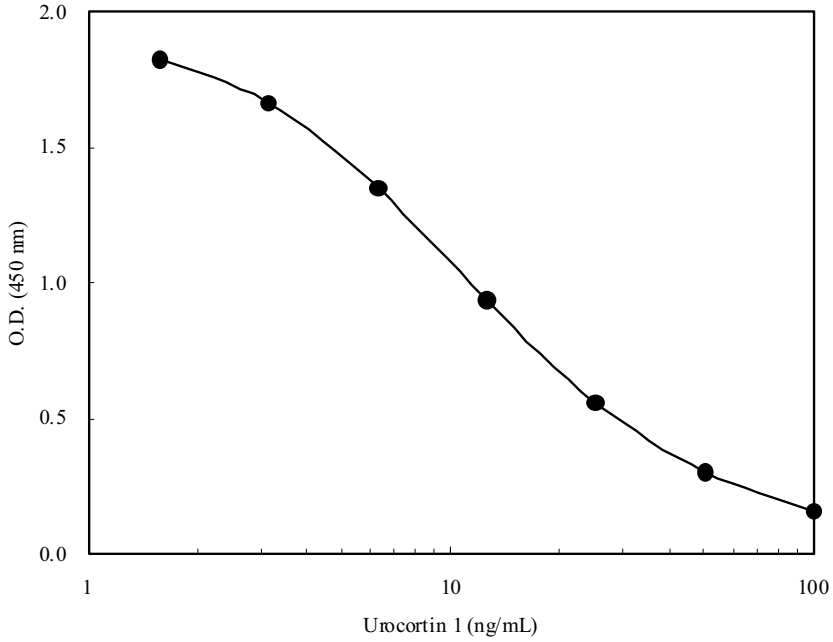
9. For accurate quantification, always run standard curve when testing samples.

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.

Ⅵ. Performance Characteristics

Typical standard curve



<Analytical recovery>

<Rat Serum A>

Added Urocortin 1 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	4.34		
2.0	6.62	6.34	104.42
7.0	12.64	11.34	111.46
20.0	28.45	24.34	116.89

<Rat Serum B>

Added Urocortin 1 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	2.61		
2.0	4.24	4.61	91.97
7.0	7.88	9.61	82.00
20.0	22.51	22.61	99.56

<Rat Serum C>

Added Urocortin 1 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	2.92		
2.0	4.61	4.92	93.70
7.0	7.99	9.92	80.54
20.0	22.29	22.92	97.25

<Rat Plasma A>

Added Urocortin 1 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	3.03		
2.0	5.52	5.03	109.74
7.0	9.55	10.03	95.21
20.0	20.46	23.03	88.84

<Rat Plasma B>

Added Urocortin 1 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	3.03		
2.0	5.11	5.03	101.59
7.0	9.29	10.03	92.62
20.0	19.43	23.03	84.37

<Rat Plasma C>

Added Urocortin 1 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	2.55		
2.0	4.55	4.55	100.00
7.0	7.87	9.55	82.41
20.0	20.01	22.55	88.74

<Mouse Serum A>

Added Urocortin 1 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	4.79		
2.0	6.54	6.79	96.32
7.0	11.00	11.79	93.30
20.0	24.79	24.79	100.44

<Mouse Serum B>

Added Urocortin 1 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	4.25		
2.0	6.10	6.25	97.60
7.0	10.68	11.25	94.93
20.0	25.01	24.25	103.13

<Mouse Serum C>

Added Urocortin 1 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	4.07		
2.0	5.99	6.07	98.68
7.0	10.29	11.07	92.95
20.0	27.01	24.07	112.21

<Mouse Plasma A>

Added Urocortin 1 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	5.09		
2.0	7.22	7.09	101.83
7.0	13.06	12.09	108.02
20.0	29.60	25.09	117.98

<Mouse Plasma B>

Added Urocortin 1 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	4.41		
2.0	6.39	6.41	99.69
7.0	11.27	11.41	98.77
20.0	28.22	24.41	115.61

<Mouse Plasma C>

Added Urocortin 1 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	4.24		
2.0	6.88	6.24	110.26
7.0	11.90	11.24	105.87
20.0	28.20	24.24	116.34

<Dilution test>

<Rat Serum>

Dilution Ratio	Rat A (ng/mL)	Rat B (ng/mL)
x 1	4.77	3.15
x 1.5	3.50	2.87
x 2	2.34	1.84
x 3	1.83	0.83

<Rat Plasma>

Dilution Ratio	Rat A (ng/mL)	Rat B (ng/mL)
x 1	2.63	3.41
x 1.5	2.08	2.47
x 2	1.37	1.60
x 3	0.99	1.07

<Mouse Serum>

Dilution Ratio	Mouse A (ng/mL)	Mouse B (ng/mL)
x 1	5.15	4.31
x 1.5	3.86	3.86
x 2	3.65	2.83
x 3	2.25	1.50

<Mouse Plasma>

Dilution Ratio	Mouse A (ng/mL)	Mouse B (ng/mL)
x 1	5.65	5.10
x 1.5	4.20	3.47
x 2	3.22	2.98
x 3	2.66	2.17

<Crossreactivity>

Related peptides	Crossreactivity (%)
Urocortin 1 (mouse, rat)	100.0
Urocortin 1 (human)	51.3
Urocortin 2 (mouse)	0
Urocortin 2 (rat)	0
Urocortin 3 (mouse, rat)	0
ACTH (mouse, rat)	0
ACTH (human)	0
CRF (mouse, rat, human)	0

<Precision and reproducibility>

Test sample	Intra-assay CV (%)	Inter-assay CV (%)
Rat serum	2.87-9.48	4.44-7.76
Rat plasma	1.70-13.01	5.71-15.72
Mouse serum	3.51-5.73	5.45-9.83
Mouse plasma	3.14-5.32	8.70-10.12

Ⅶ. Stability and Storage

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

< Shelf life > This kit is stable under the condition indicated for 6 months from the date of manufacturing. The expiry date is indicated on the label of the kit.

< Package > For 96 tests per one kit including standards

Ⅷ. References

1. Vaughan J, Donaldson C et al (1995): Urocortin, a mammalian neuropeptide related to fish urotensin I and to corticotropin-releasing factor. *Nature* **378,** 287-292.
2. Donaldson CJ, Sutton SW et al (1996): Cloning and characterization of human urocortin. *Endocrinology* **137,** 2167-2170.
3. Zhao L, Donaldson CJ et al (1998): The structures of the mouse and human urocortin genes (Ucn and UCN). *Genomics* **50,** 23-33
4. Oki Y, Iwabuchi M et al (1998): Distribution and concentration of urocortin, and effect of adrenalectomy on its contents in rat hypothalamus. *Life Sci* **62,** 807-812
5. Koziez F, Yanaihara H et al (1998): Distribution of urocortin-like immunoreactivity in the central nervous system of the rat. *Comp Neurol* **391,** 1-10
6. Dautzenberg FM, Kilpatrick GJ et al (2001): Molecular biology of the CRH receptors in the mood. *Peptides* **22,** 753-760
7. Davis ME, Pemberton CJ et al (2004): Urocortin-1 infusion in normal humans. *J Clin Endocrinol Metab* **89,** 1402-0409
8. Ng Li, Loke IW et al (2004): Plasma urocortin in human systolic heart failure. *Clin Sci (Lond)* **106,** 383-388

Manufactured by Yanaihara Institute Inc.

COSMO BIO CO., LTD.
Inspiration for Life Science
TOYO 2CHOME, KOTO-KU, TOKYO, 135-0016, JAPAN
http://www.cosmobio.co.jp e-mail : export@cosmobio.co.jp
Phone : +81-3-5632-9617 FAX : +81-3-5632-9618