Mouse/rat Urocortin 3 ELISA

Cat. No.: RSCYK200R

1. Introduction

Urocortin 3 (Ucn3) or stresscopin (SCP) is a new member of the corticotropin-releasing factor (CRF) peptide family identified in the mouse and human.[1] The CRF family of neuropeptides includes mammalian peptides CRF, urocortin 1(Ucn1) and urocortin 2 (Ucn2) or stress-related peptide (SRP), as well as piscine urotensin 1 and frog sauvagine. Mouse and human Ucn3 share 90% identity in the 38-aa putative mature peptide.

In the human, Ucn1 immunoreactivity was marked in the medulla, whereas Ucn3 was immunostained mostly in the cortex. [2] The receptors for Ucn1, Ucn2, Ucn3 and CRF are all expressed in human adrenal cortex and medulla[2], therefore these peptides are expected to play important roles in physiological adrenal functions.[2] Ucn3 was also detected by RIA in human heart 0.74-1.15 pmol/g wet weight, kidney 1.21 pmol /g wet weight, pituitary 2.72 pmol /g wet weight and brain tissues 1-2 pmol /g wet weight.[3] Furthermore, immunoreactive Ucn3 was present in human plasma 51.8 pmol/L and urine 266 pmol/L obtained from healthy subjects.[3] It was also detected in human rectum 15.4 pmol/g wet weight and sigmoid colon 6.5 pmol/g wet weight.[4] These data suggest that Ucn3 regulates the cardiac and renal functions as a local factor and a circulating hormone and plays some physiological or pathological roles in the modulation of gastrointestinal functions during stressful conditions in different manners from those of Ucn1.[4]

Pharmacological studies showed that Ucn3 is a high-affinity ligand for the type 2 CRF receptor (CRFR2). In the rat, Ucn3-positive neurons were found predominantly within the hypothalamus and medial amygdala.[5] Ucn3 fibers were distributed mainly in the hypothalamus and limbic structures.[5] These data support that Ucn3 is an endogenous ligand for CRFR2 in these areas. The results also suggest that Ucn3 is positioned to play a role in mediating physiological functions, including food intake and neuroendocrine regulation.[5]

In the mouse, Ucn3 was expressed in pancreatic beta-cells and in a mouse beta cell line, MIN6. High potassium, forskolin or high glucose could stimulate Ucn3 secretion from these cells.[6] Ucn3 injections to the rat resulted in significant increase of plasma insulin level.[6] Ucn3 also stimulated glucagon and insulin release from isolated rat islets.[6] Pancreatic Ucn3 acting through CRFR2 was suggested to be involved in the local regulation of glucagon and insulin secretion.[6]

Treatment with Ucn3 (SCP) or Ucn2 (SRP) suppressed food intake, delayed gastric emptying and decreased heat-induced edema.[7] Thus Ucn3 (SCP) and Ucn2 (SRP) might represent endogenous ligands for maintaining homeostasis after stress, and could allow the design of drugs to ameliorate stress-related diseases.[7] The use of CRFR2 selective agonists, Ucn2 and Ucn3, to treat ischemic heart disease was proposed because of their potent cardioprotective effects in murine heart and their minimal impact on the hypothalamic stress axis.[8]

Ucn1 is able to bind to two types of G-protein coupled receptors: CRFR1 and CRFR2, whereas Ucn3 (SCP) and Ucn2 (SRP) bind exclusively and with high affinity to CRFR2. [9] Ucn3 (SCP) is expressed in rat cardiomyocytes and the levels of Ucn3 (SCP) and Ucn2 (SRP) were increased by hypoxic stress.[8] All these three peptide were shown to have potent cardioprotective effects in cells exposed to hypoxia/reoxygenation.[9]

We have already developed mouse urocortin 2 EIA kit (YK190), and this time urocortin 3 EIA kit (YK200) is been developing in our laboratory which highly specific for mouse/rat urocortin 3 with almost no cross reaction to Ucn1 (mouse, rat), Ucn1 (human), Ucn2 (mouse), Ucn2 (rat), ACTH (mouse, rat), ACTH (human) and CRF (mouse, rat, human). The kit can be used for measurement of Ucn3 in mouse/rat plasma, serum and their brain tissue extracts with high sensitivity (The brain tissue extracts need to be treated with solid-phase extraction cartridges). It will be a specifically useful tool for Ucn3 researches.

RSCYK200R Mouse/Rat Urocortin 3 EIA Kit	**Contents**
▼ The assay kit can measure Mouse/rat Urocortin 3 in the range of 0.41-100 ng/mL	1) Antibody coated plate
▼ The assay completes within 16-18 hr.+ 3 hr.	2) Standard
▼ With one assay kit, 41 samples can be measured i duplicate	3) Labeled antigen
▼ Test sample: Mouse/rat plasma and serum, brain tissue extracts (The brain tissue extracts need to be treated with solid-phase extraction catrtridges). Sample volume 25 µL	4) SA-HRP solution 5) Substrate buffer
▼ The 96-well plate in kit was consisted by 8-wells strips. The kit can be used separately.	6) OPD tablet
▼ Stability and Storage	7 Stopping solution
Store all the components at 2-8°C.	8) Buffer solution
The kit is stable undet the condition for 15 months From the date of manufacturing..	9) Washing solution (concentrated)
The expiry date is indicated on the label of kit.	10) Adhesive foil

2. Characteristics

This EIA kit is used for quantitative determination of urocortin 3 in mouse/rat plasma, serum and their brain tissue extracts. The kit is characterized for sensitive quantification, high specificity and no influence with other components in samples. Mouse/rat urocortin 3 standard is highly purified synthetic product.

Specificity

The EIA kit has high specificity to mouse/rat urocortin 3 and shows cross reactivity neither Ucn1 (mouse, rat), Ucn1 (human), Ucn2 (mouse), Ucn2 (rat), ACTH (mouse, rat), ACTH (human) nor CRF (mouse, rat, human).

Assay Principle

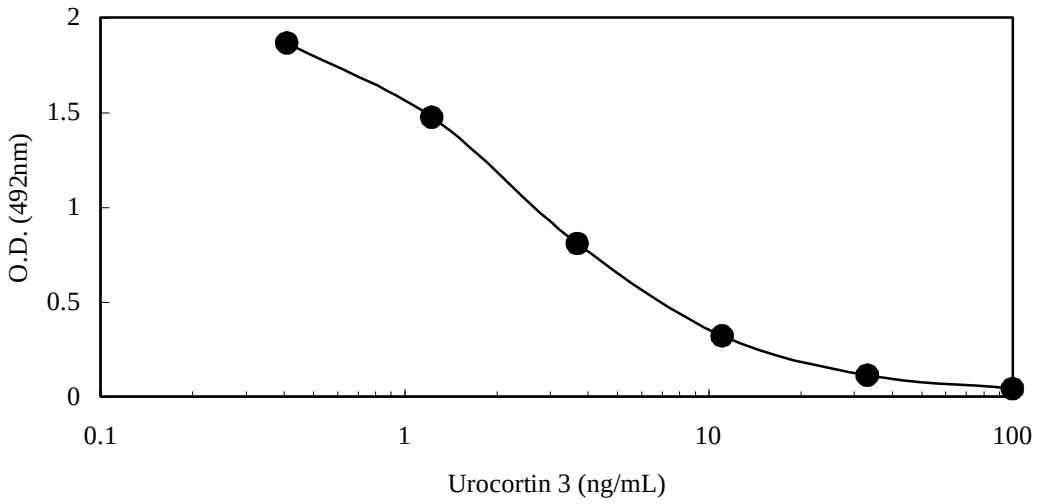
This EIA kit for determination of mouse/rat urocortin 3 in samples is based on a competitive enzyme immunoassay using combination of highly specific antibody to mouse/rat urocortin 3 with biotin-avidin affinity system. The 96 wells plate is coated with rabbit anti mouse/rat urocortin 3 antibody. Mouse/rat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

3. Mouse and rat brain tissue extraction and preparation

1. Materials:
   Mouse or rat brain tissue
   Extraction buffer: PBS containing 0.2% Nonidet P-40
   Extraction column: Oasis HLB 3cc (60mg) extraction cartridge (part No.WAT094226, Waters)
   Extraction maniholde (Waters), Centrifugal vaporizer (CVE-200D, EYELA, Japan), plastic tubes and glass tubes, methanol (HPLC grade), distilled water, homogenizer
   Elution buffer: Acetonitrile-0.075%TFA (80:20,vol/vol)
2. Mouse or rat brain tissue is weighed and then homogenized in 15–fold volume of extraction buffer in an ice bath. The homogenate is centrifuged in plastic tubes (15,000 rpm/min, 20 min) at 4°C, and the supernatant is transferred into a glass tube in an ice bath.
3. Methanol (6mL) is applied onto an extraction column for conditioning, and then drained by aspiration (2 mL/min). The column is equilibrated twice with distilled water (3mL each) and the supernatant above mentioned is applied onto the column with a pipette (for example 2 mL). The volume of the supernatant applied should be recorded. The column is aspirated slowly then washed twice with distilled water (3 mL each) and finally eluted with elution buffer (2 mL). The eluate is collected in a glass tube and dried in a centrifugal vaporizer. The mouse or rat brain extracts (dry residue) should be used as soon as possible after drying. If the dry residue is tested later, they should be stored at or below –30ºC until assay.
4. The dry residue (sample for assay) is reconstituted with buffer solution in kit (75% volume of supernatant volume applied onto the column that recorded, for example 1.5 mL). The insoluble material should be removed by centrifugation （3,000 rpm/min,15 min) at 4ºC and the sample solution is submitted to assay immediately.

4. Performance Characteristics

Typical standard curve



Analytical recovery

Mouse Plasma A

Added Urocortin 3 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.38		
1.0	1.10	1.38	79.71
5.0	4.27	5.38	79.37
30.0	21.97	30.38	72.32
50.0	53.77	50.38	106.73

Mouse Plasma B

Added Urocortin 3 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.26		
1.0	1.06	1.26	84.13
5.0	4.77	5.26	90.68
30.0	26.05	30.26	86.09
50.0	46.42	50.26	92.36

Mouse Plasma C

Added Urocortin 3 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.31		
1.0	1.12	1.31	85.50
5.0	4.22	5.31	79.47
30.0	26.41	30.31	87.13
50.0	49.52	50.31	98.43

Mouse Plasma D

Added Urocortin 3 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.34		
1.0	1.08	1.34	80.60
5.0	4.24	5.34	79.40
30.0	22.40	30.34	73.83
50.0	52.38	50.34	104.05

Mouse Serum A

Added Urocortin 3 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.77		
1.0	1.32	1.77	74.58
5.0	5.63	5.77	97.57
30.0	25.91	30.77	84.21
50.0	45.58	50.77	89.78

Mouse Serum B

Added Urocortin 3 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.40		
1.0	1.74	1.40	124.29
5.0	5.66	5.40	104.81
30.0	25.68	30.40	84.47
50.0	38.73	50.40	76.85

Mouse Serum C

Added Urocortin 3 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.43		
1.0	1.31	1.43	91.61
5.0	5.55	5.43	102.21
30.0	27.46	30.43	90.24
50.0	35.78	50.43	70.95

Mouse Serum D

Added Urocortin 3 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.46		
1.0	1.42	1.46	97.26
5.0	5.27	5.46	96.52
30.0	27.84	30.46	91.40
50.0	37.87	50.46	75.05

Rat Plasma A

Added Urocortin 3 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.32		
1.0	1.48	1.32	112.12
5.0	4.90	5.32	92.11
30.0	27.43	30.32	90.47
50.0	52.62	50.32	104.57

Rat Plasma B

Added Urocortin 3 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.58		
1.0	1.41	1.58	89.24
5.0	5.31	5.58	95.16
30.0	29.84	30.58	97.58
50.0	56.69	50.58	112.08

Rat Plasma C

Added Urocortin 3 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.54		
1.0	1.56	1.54	101.30
5.0	4.88	5.54	88.09
30.0	30.88	30.54	101.11
50.0	64.49	50.54	127.60

Rat Plasma D

Added Urocortin 3 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.79		
1.0	1.81	1.79	101.12
5.0	5.41	5.79	93.44
30.0	28.68	30.79	93.15
50.0	66.94	50.79	131.80

Rat Serum A

Added Urocortin 3 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.17		
1.0	1.18	1.17	100.85
5.0	4.03	5.17	77.95
30.0	27.80	30.17	92.14
50.0	61.76	50.17	123.10

Rat Serum B

Added Urocortin 3 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.21		
1.0	1.04	1.21	85.95
5.0	4.80	5.21	92.13
30.0	28.89	30.21	95.63
50.0	62.71	50.21	124.90

Rat Serum C

Added Urocortin 3 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.27		
1.0	1.15	1.27	90.55
5.0	4.50	5.27	85.39
30.0	27.48	30.27	90.78
50.0	73.87	50.27	146.95

Rat Serum D

Added Urocortin 3 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.14		
1.0	1.03	1.14	90.35
5.0	3.81	5.14	74.12
30.0	23.14	30.14	76.78
50.0	59.77	50.14	119.21

Mouse Brain

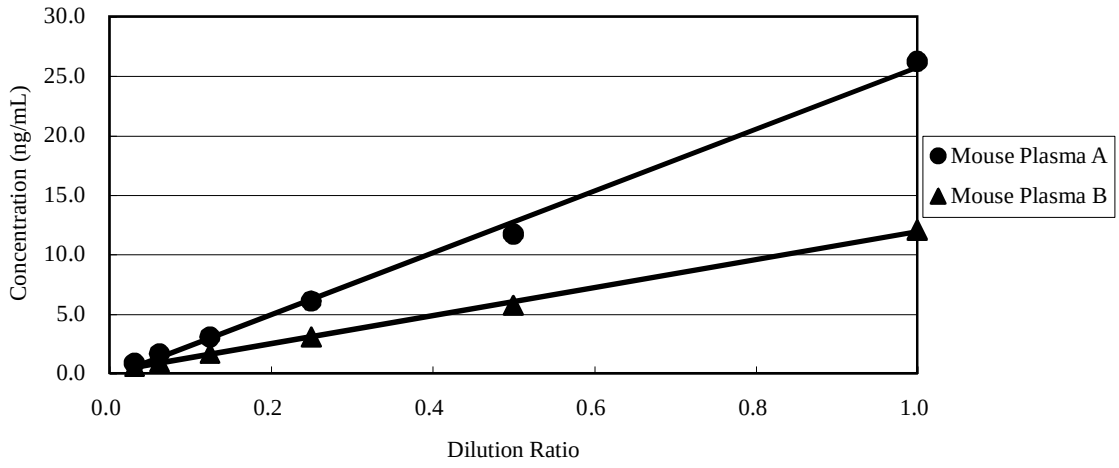
Added Urocortin 3 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.27		
1.0	0.80	0.77	103.90
5.0	4.95	5.27	93.93
30.0	31.17	30.27	102.97

Rat Brain

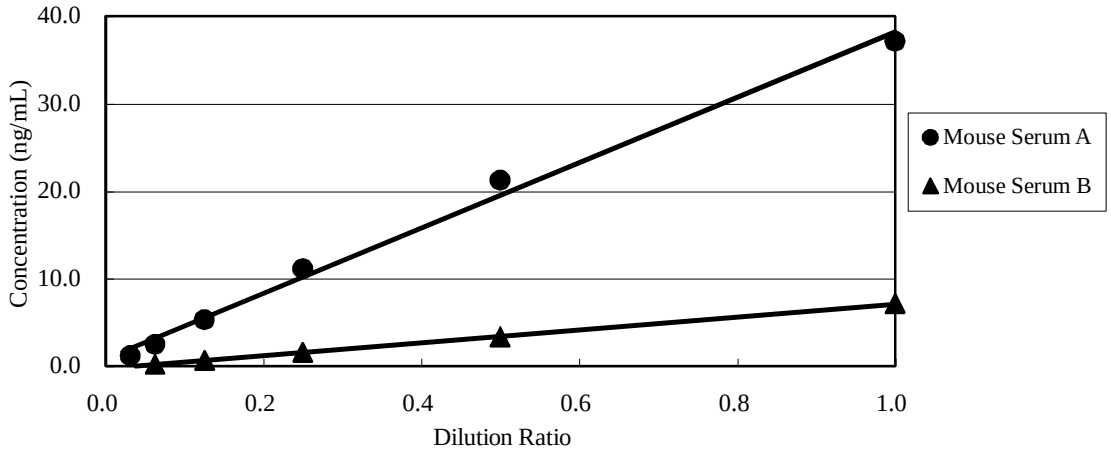
Added Urocortin 3 (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.22		
1.0	0.69	0.72	95.83
5.0	4.24	5.22	81.23
30.0	26.93	30.22	89.11

Dilution Test

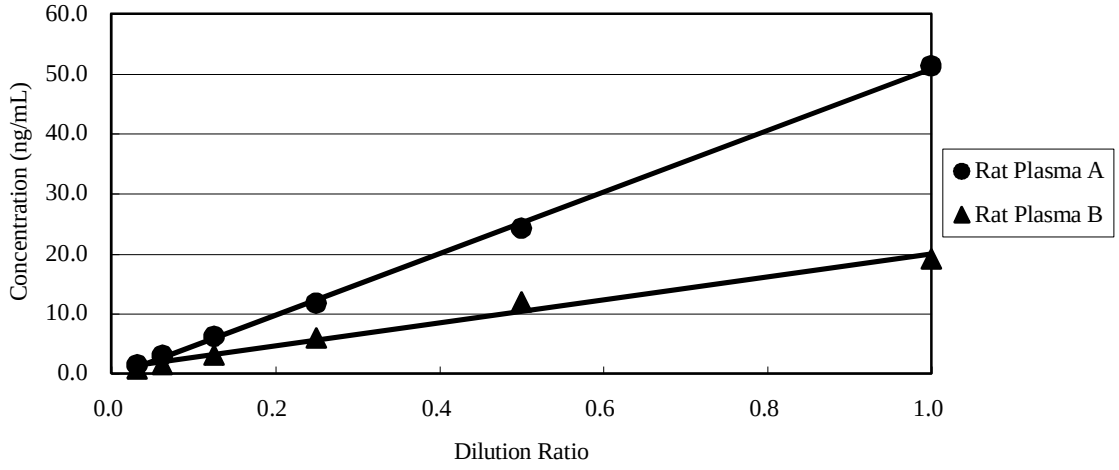
Mouse Plasma



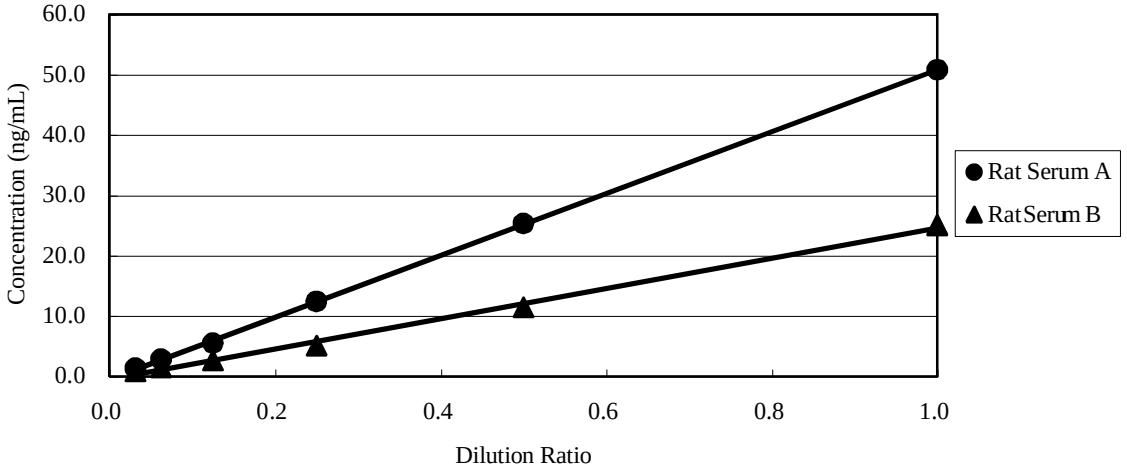
Mouse Serum



Rat Plasma



Rat Serum



Crossreactivity

Related peptides	Crossreactivity (%)
Urocortin 3 (mouse, rat)	100
Urocortin 1 (mouse, rat)	0
Urocortin 1 (human)	0.04
Urocortin 2 (mouse)	0
Urocortin 2 (rat)	0
ACTH (mouse, rat)	0.03
ACTH (human)	0.03
CRF (mouse, rat, human)	0.01

Precision and reproducibility

Test Sample	Intra-assay CV(%)	Inter-assay CV(%)
Mouse Plasma	6.13-12.35	2.50- 9.33
Mouse Serum	5.10-13.58	5.69-10.24
Rat Plasma	10.51-15.50	14.62-23.42
Rat Serum	8.32-13.15	11.29-16.93

5. Stability and Storage

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

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

Package For 96 tests per 1 kit including standards

6. References

1. Lewis K, Li C et al: (2001) Identification of urocortin III, an additional member of the corticotropin-releasing factor (CRF) family with high affinity for the CRF2 receptor. *Proc Natl Acad Sci USA.* ***98***, 7570-7575
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4. Saruta M, Takahashi K et al: (2005) Urocortin3/stresscopin in human colon: possible modulators of gastrointestinal function during stressful conditions. *Peptide.* ***26***, 1196-1206
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