KAMIYA BIOMEDICAL COMPANY

Mouse and Rat Urocortin 1 EIA

For the quantitative determination of Urocortin 1 in mouse and rat plasma and serum.

Cat. No. KT-628

For Research Use Only.

PRODUCT INFORMATION

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INTENDED USE
The mouse and rat Urocortin 1 is for the quantitative determination of Urocortin 1 in mouse and rat plasma and serum. For research use only.

INTRODUCTION
Urocortin 1 (Ucn 1) was first identified in rats, and later in humans and mice. It is the second mammalian member of the CRF family. Rat and mouse Ucn1 have the same amino acid sequence and displays 95% structure homology to human Ucn 1, 45% to CRF, and 63% to urotensin. In rat, Ucn 1 immunoreactivity (IR) was shown to distribute widely in the central nervous system, endocrine organs, and digestive system, and its highest concentration was in pituitary (11 pmol/g, w.w.). A polyclonal antibody was used against rat Ucn 1 to define the distribution of Ucn 1-IR in the rat central nervous system and found a large number of neurons with Ucn 1-IR in rat brain.

Synthetic human Ucn 1 binds with high affinity to CRF receptor type 1 (CRFR1), 2 alpha (CRFR2alpha), and 2 beta (CRFR2beta). 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. Data supports the hypothesis that this peptide is the endogenous ligand for CRFR2.

Synthetic human Ucn 1 stimulates cAMP accumulation in cells stably transfected with those receptors and acts in vivo to release ACTH from dispersed rat anterior pituitary cells. In addition, the CRF-binding protein binds human Ucn 1 with high affinity and can prevent Ucn 1 stimulated ACTH secretion in vitro. Ucn 1 was suggested to play an important rol in various tissues in normal rats, but has not shown to behave as a hypothalamic hypophysiotropic factor in mediating adrenocorticotropin seretin in adrenalectomized rats. Ucn 1 has been implicated in various endocrine responses, such as blood pressure regulation, as well as in higher cognitive functions.

Synthetic human Ucn 1 also stimulates plasma ACTH, cortisol, and atrial natriuretic peptide (ANP) secretin and suppresses plasma ghrelin in healthy male volunteers. In the human, plasma Ucn 1 is elevated in heart failure, especially in its early stages. This fact may be useful in the diagnosis of early heart failure.

This ELISA is highly specific for mouse/rat Ucn 1 with almost no crossreaction with Ucn 2 (mouse/rat), Ucn 3 (mouse/rat), ACTH (mouse/rat/human), and CRF (mouse/rat/human). The kit can be used for measurement of Ucn 1 in mouse and rat serum or plasma with high sensitivity.

Mouse/Rat Urocortin 1 EIA Kit

- The assay kit can measure mouse/rat urocortin 1 in mouse/rat plasma and serum 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: Mouse/rat plasma and serum
  Sample volume: 10 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 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.

Contents

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

CHARACTERISTICS

This EIA kit is used for the quantitative determination of urocortin 1 in mouse/rat serum and plasma samples. The kit is characterized by its sensitive quantification and its high specificity. The mouse/rat urocortin 1 in mouse/rat calibrator is a highly purified synthetic product.

Specificity

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

Principal

This kit for the determination of mouse and rat urocortin 1 in samples is based on a competitive enzyme immunoassay using a combination of highly specific antibodies to mouse and rat urocortin 1 and a biotin-avidin affinity system. The calibrator or samples, and the labeled antigen are added to the pre-coated 96-well plate are added for competitive immunoreaction. After incubation and 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 addition of TMB and the concentration of the mouse or rat Ucn 1 is calculated.

COMPONENTS

Component	Form	Quantity	Main Ingredient
1. Antibody-Coated Plate	MTP[*1]	1 plate (96-well)	Rabbit anti-mouse and rat Urocortin 1 antibody
2. Urocortin 1 Calibrator	Lyophilized	1 vial (100 ng)	Synthetic mouse and rat Urocortin 1
3. Labeled Antigen	Lyophilized	1 vial	Biotinylated mouse and rat Urocortin 1
4. SA-HRP Solution	Liquid	1 bottle (12 mL)	HRP-labeled streptoavidin
5. Enzyme-Substrate Soln.	Liquid	1 bottle (12 mL)	3, 3', 5, 5' -Tetramethylbenzidine
7. Stop Solution	Liquid	1 bottle (12 mL)	1 M H_2SO_4
8. Buffer Solution	Liquid	1 bottle (15 mL)	Tris-HCL 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 450 nm
- Microtiter plate shaker
- 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

REAGENT PREPARATION

1. Preparation of Calibrator Solutions: Reconstitute the Urocortin 1 Calibrator with 1 mL of Buffer Solution, giving a 100 ng/mL Calibrator Solution after reconstitution. 0.1 mL of the reconstituted Calibrator Solution is diluted with 0.1 mL of Buffer Solution to yield a 50 ng/mL Calibrator Solution. Repeat the serial dilution to make Calibrator Solutions at 25, 12.5, 6.25, 3.125, and 1.563 ng/mL. Buffer Solution is used as the zero calibrator (0 ng/mL).

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

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

4. Other reagents are ready for use.

ASSAY PROTOCOL

1. Warm the reagents and samples to room temperature (20 - 30℃) before beginning the test.
2. Add 0.3 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 40 µL Buffer Solution into the wells, then add 10 µL of the prepared Calibrator Solutions (0, 1.563, 3.125, 6.25, 12.5, 25, 50, and 100 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℃ for 16-18 hours. Keep still, 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 0.3 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 0.3 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 30 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 450 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 unkonwn 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.

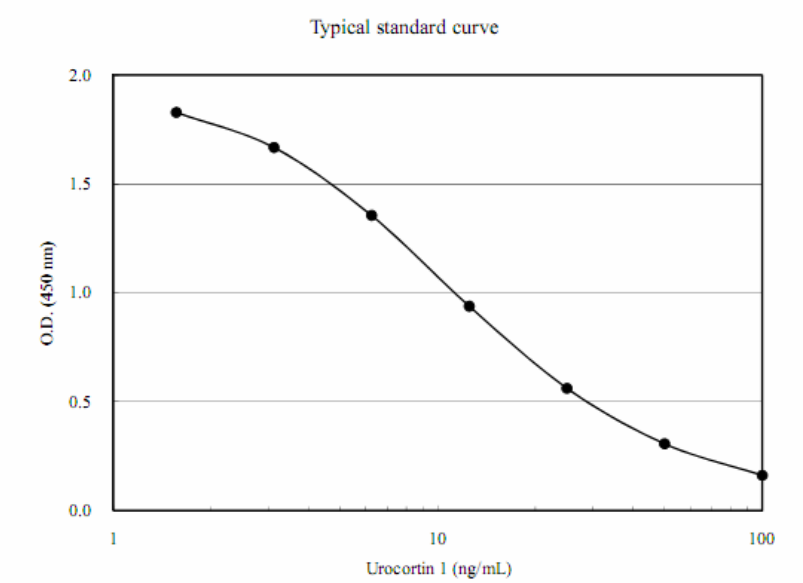
When a sample value exceeds 100 ng/mL, it must be diluted with Buffer Solution and re-assayed until the sample value is within the assay range.

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 samples are tested later, they should be aliquoted and frozen at or below -30℃.
2. Calibrator 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 should be stored at or below -30℃ (stable for 1 month).
3. The total pipetting time of calibrator solutions and samples for the whole plate should not exceed 30 minutes.
4. During storage of washing solution (concentrated) at 4℃, precipitates may be observed, however they will be dissolved when diluted. Diluted wash solution is stable for 6 months at 4℃.
5. Pipetting operations may affect the precision of the assay, so pipette all solutions precisely. In addition, use clean test tubes or vessels in assay and use a new tip for each calibrator 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 with a plate shaker to promote immunoreaction (approximately 100 rpm).
8. Perform all the determination in duplicate.
9. Read plate optical absorbance in wells as soon as possible after adding the stop solution.
10. To quantitate accurately, always run a calibration curve when testing samples.
11. Protect reagents from strong light (e.g. direct sunlight) during storage and assay.
12. Do not mix lots.
13. Some reagents contain human serum (tested and found negative for HBsAG, HIV ½, HCV, HIV-1 AG or HIV-1 NAT, ALT and Syphilis by FDA approved methods), care should be taken when handling.

Performance Characteristics



<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

Assay Range
1.563-100 ng/mL

Storage
Store all components at 2-8℃. Kit is stable until expiration date.

FOR RESEARCH USE ONLY

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