YK132 Human CRF ELISA

FOR LABORATORY USE ONLY

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

YK132 Human CRF ELISA Kit

Ⅰ. Introduction

Corticotropin releasing factor (CRF, also CRH) was initially isolated from ovine hypothalamus by Vale et al., in 1981, and identified as a novel neuropeptide comprising 41 amino acid residues with molecular weight 4758 [1]. Later human CRF[2] and rat CRF[3] were also isolated and identified. The mouse CRF peptide is identical at amino acid level to the rat and human CRF peptides[4]. CRF in anterior pituitary promotes the synthesis and secretion of ACTH, a main factor of hypothalamus-pituitary-adrenal (HPA) axis. In the rat and human, CRF distributes mainly in hypothalamus, but it was also found in spinal cord, stomach, spleen, duodenum, adrenal and placenta. In addition, immunochemical evidence supported the wide distribution of the peptide throughout the central nervous system (CNS) such as olfactory bulb, retina and central auditory system in the rat.

In mouse brain extracts, the highest concentrations of CRF-like immunoreactivity (CRF-LI) has been detected in median eminence and hypothalamus and also existing in amygdala, thalamus, frontal cortex, medulla/pons and cerebellum by radioimmunoassay[5]. However because of the wide distribution, it is still disputing about CRF whether its blood level can reflect only the function of HPA axis [6].

The relationships between CRF and stress, CRF and Alzheimer disease (AD) were attracted much attention recently. In fact the peptide was also suggested to regulate endocrine, autonomic and behavioral responses to stress, based on an experiment with acute and chronic stress rat models that showed endocrine function changes similar to those seen in patients with depression [6] CRF in serial cerebrospinal fluid（CSF）of patients with depression was strikingly reduced as compared to those of normal subjects [7,8] . The mean CRF and ACTH levels in the CSF of AD patients were significantly lower than those of healthy controls [9]. Only in the cortices of those with mild dementia, CRF was reduced significantly. Thus CRF was proposed to serve as a potential neurochemical marker of early dementia and possibly early AD [10].

A large proportion of CRF in human brain was shown to be in the form of complex with its binding protein (CRF-BP). CRF molecule in the complex is unavailable for activation of the CRF receptor. Accordingly reduction in total CRF do not necessarily predict reduction of bioactive free CRF, and the levels of total CRF and CRF in the form of complex (CRF/CRF-BP) were suggested to be the main factors determining the quantity of bioactive free CRF in human brain [11]. In AD there have been observed dramatic reduction in the content of free CRF in brain and thus displacement of CRF from CRF-BP was proposed as a possible treatment for AD [12] . In primary neuron culture, CRF exhibited protective effect against cell death induced by amyloid-beta peptide, suggesting that disturbances in HPA axis function can occur independently of alteration in CRF mRNA levels in AD brain and further suggesting an additional role for CRF in protecting neurons against cell death [13]. On the other hand, Yanaihara et al. demonstrated immunoreative CRF in various neuroendocrine tumors, and suggested that the blood level of the peptide might be used as a tumor marker [14] .

YK132 Human CRF ELISA Kit

- ▼ The assay kit can measure human CRF within the range of 0.078-2.5 ng/mL.
- ▼ The assay is completed within 7.5 hr.
- ▼ With one assay kit, 41 samples can be measured in duplicate.
- ▼ Test sample: Human plasma (Pretreatment of plasma before assay is necessary)
  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 (%)
  Human plasma 1.17-4.34
  Inter-assay CV (%)
  Human plasma 1.46-9.15
- ▼ Stability and storage
  Store all of the components at 2-8℃.
  The kit is stable under the condition for 24 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 antibody solution
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 ELISA kit is used for quantitative determination of human CRF in plasma (Pretreatment of plasma before assay is necessary). The kit is characterized by its sensitive quantification and high specificity. In addition, it has no influence by other components in samples. CRF standard is highly purified synthetic product.

< Specificity >

This ELISA kit has high specificity to CRF, and shows no crossreactivity to ACTH, urocortin 1, urocortin 2 (mouse) and urocortin 3 (mouse, rat). The detail data are presented on page 11.

< Assay principle >

This ELISA kit for determination of human CRF is based on a sandwich enzyme immunoassay. To the wells of plate coated with highly purified antibody against CRF, standards or samples are added for the 1st step immunoreaction. After the 1st step incubation and plate washing, labeled antibody solution (biotinylated rabbit anti CRF antibody) is added as the 2nd step to form antibody - antigen - labeled antibody complex on the surface of the wells. After the 2nd step incubation and rinsing out excess labeled antibody, horseradish peroxidase (HRP) labeled streptoavidin (SA) is added for binding to labeled antibody. Finally, HRP enzyme activity is determined by 3,3',5,5'-Tetramethylbenzidine (TMB) and the concentration of human CRF is calculated.

III. Composition

	Component	Form	Quantity	Main Ingredient
1.	Antibody coated plate	microtiter plate	1 plate (96 wells)	Rabbit anti CRF antibody coated
2.	Standard	lyophilized	1 vial (2.5 ng)	Synthetic CRF (1-41)
3.	Labeled antibody solution	liquid	1 bottle (12 mL)	Biotinylated rabbit anti CRF antibody
4.	SA-HRP solution	liquid	1 bottle (12 mL)	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 solution	liquid	1 bottle (25 mL)	Buffer containing a reaction accelerator
8.	Washing solution (concentrated)	liquid	1 bottle (50 mL)	Concentrated saline
9.	Adhesive foil		4 pieces	

Ⅳ. Method

< Equipment required >

1. Photometer for microtiter plate (plate reader) which can read extinction 2.5 at 450nm
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

<Preparation of assay sample>

Extraction method of human plasma:
Materials: Human plasma, 95% ethyl alcohol
Methods:

1) Add plasma sample to each microfuge tubes (1.5 mL – 2.0 mL size) in an ice bath.
2) Add 3.7-fold volume of 95% ethyl alcohol in each tube. Cap tubes tightly, invert tubes and vortex well immediately.
3) Incubate tubes in an ice bath for 30 minutes.
4) Invert tubes to mix, then centrifuge at 10,000 rpm for 10 minutes (4℃) in a microfuge.
5) Decant supernatants into glass tubes. Stopper the tubes with a rubber stopper with an 18 gauge needle inserted.
6) Place tubes into a centrifugal vacuum evaporator for dry down. Dry the tubes for complete dryness at room temperature.

Reconstitute samples with kit buffer solution (same volume with added human plasma). Vortex gently until samples has dissolved into solution. The samples are now ready to be assayed directly in these tubes.

< Preparatory work >

1. Preparation of standard solution:
   Reconstitute the CRF standard with 1 mL of buffer solution, which affords 2.5 ng/mL standard solution. The reconstituted standard solution (0.2 mL) is diluted with 0.2 mL of buffer solution that yields 1.25 ng/mL standard solution. Repeat the dilution procedure to make each standard solution of 0.625, 0.313, 0.156 and 0.078 ng/mL. Buffer solution itself is used as 0 ng/mL standard solution.
   If a sample concentration below 0.078 ng/mL is predicted, standard curve may be further set up a lower detection limit by using 0.039 ng/mL standard solution which can be prepared by 2-fold dilution of 0.078 ng/mL standard solution. In such case, however, assay precision may not be so excellent as that of the cases between 0.078 and 2.5 ng/mL.

2. Preparation of washing solution:
   Dilute 50 mL of washing solution (concentrated) to 1,000 mL with distilled or deionized water.

3. 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 0.35 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). 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 100µL of buffer solution to the wells first, and then introduce 50µL of each of standard solutions (0, 0.078, 0.156, 0.313, 0.625, 1.25 and 2.5 ng/mL) or samples to the wells.

4. Cover the plate with adhesive foil and incubate it at room temperature for 4 hours. During the incubation, the plate should be shaken with a plate shaker (approximately 100 rpm).

5. After incubation, take off the adhesive foil, aspirate and wash the wells 4 times with 0.35 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 labeled antibody 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 0.35 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 SA-HRP solution to each of the wells.

10. Cover the plate with adhesive foil and incubate it at room temperature for 1 hour. During the incubation, the plate should be shaken with a plate shaker (approximately 100 rpm).

11. Take off the adhesive foil, aspirate and wash the wells 4 times with 0.35 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.

12. 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).

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

14. 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 (1mg/mL) additive blood collection tube is recommended for the plasma collection. 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 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 reagent (standard) should be stored at or below -30°C (stable for 1 month) .

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

4. 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.

5. When sample concentration exceeds 2.5 ng/mL, it needs to be diluted with buffer solution to proper concentration.

6. During the incubation except the color reaction, the plate should be shaken gently by a plate shaker to promote immunoreaction (approximately 100 rpm).

7. Perform all the determination in duplicate.

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

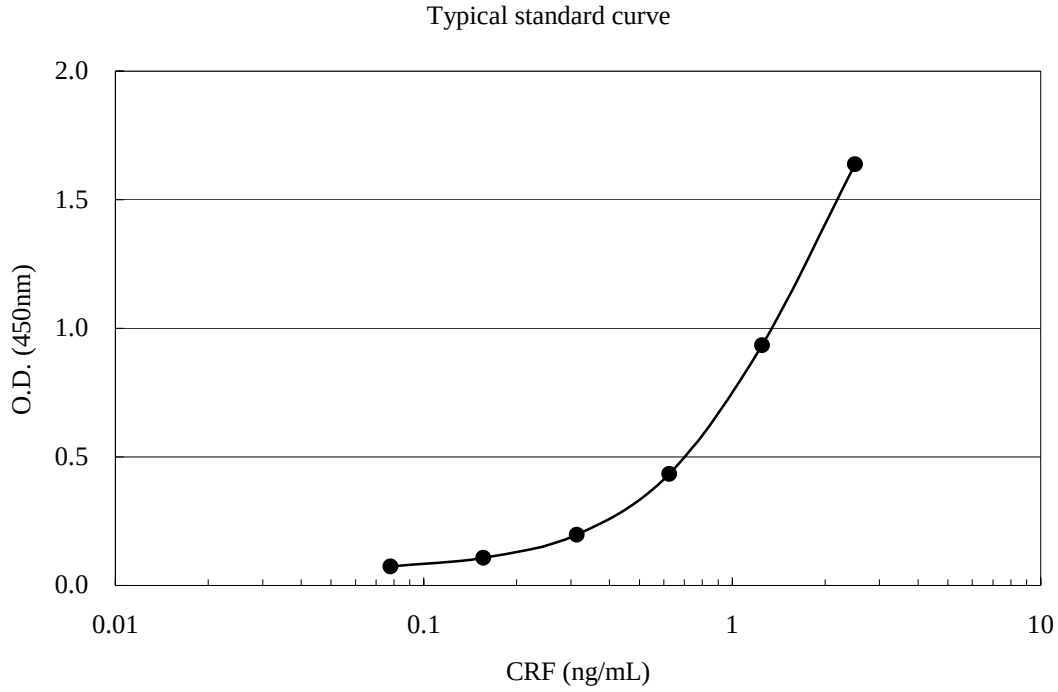
9. To quantitate accurately, always run a standard curve when testing samples.

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.

12. Some reagents contain human serum (tested and found negative for HBsAG, HIV 1/2, HCV, HIV-1 AG or HIV-1 NAT, ALT and a test for Syphilis by FDA approved methods), care should be taken when handling.

Ⅵ. Performance Characteristics



<Analytical recovery>

＜Human plasma A＞

Added CRF (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.166		
0.1	0.226	0.266	84.96
0.3	0.388	0.466	83.26
1.0	1.015	1.166	87.05

＜Human plasma B＞

Added CRF (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.149		
0.1	0.215	0.249	86.35
0.3	0.387	0.449	86.19
1.0	1.016	1.149	88.42

＜Human plasma C＞

Added CRF (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.139		
0.1	0.222	0.239	92.89
0.3	0.382	0.439	87.02
1.0	1.040	1.139	91.31

＜Human plasma D＞

Added CRF (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.159		
0.1	0.236	0.259	91.12
0.3	0.394	0.459	85.84
1.0	1.057	1.159	91.20

＜Human plasma E＞

Added CRF (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.162		
0.1	0.209	0.262	79.77
0.3	0.379	0.462	82.03
1.0	0.988	1.162	85.03

＜Human plasma F＞

Added CRF (ng/ml)	Observed (ng/ml)	Expected (ng/ml)	Recovery (%)
0.0	0.151		
0.1	0.204	0.251	81.27
0.3	0.387	0.451	85.81
1.0	1.054	1.151	91.57

<Dilution test>

＜Human plasma A＞

Sample dilution	Observed (ng/ml)	Expected (ng/ml)	% of Expected
X1	0.150	0.150	
X1.5	0.108	0.100	108.0
X2.25	0.069	0.067	103.5

＜Human plasma B＞

Sample dilution	Observed (ng/ml)	Expected (ng/ml)	% of Expected
X1	0.183	0.183	
X1.5	0.114	0.122	93.4
X2.25	0.052	0.081	63.9

＜Human plasma C＞

Sample dilution	Observed (ng/ml)	Expected (ng/ml)	% of Expected
X1	0.188	0.188	
X1.5	0.146	0.125	116.5
X2.25	0.088	0.084	105.3

＜Human plasma D＞

Sample dilution	Observed (ng/ml)	Expected (ng/ml)	% of Expected
X1	0.204	0.204	
X1.5	0.127	0.136	93.4
X2.25	0.094	0.091	103.7

＜Human plasma E＞

Sample dilution	Observed (ng/ml)	Expected (ng/ml)	% of Expected
X1	0.151	0.151	
X1.5	0.102	0.101	101.3
X2.25	0.061	0.067	90.9

＜Human plasma F＞

Sample dilution	Observed (ng/ml)	Expected (ng/ml)	% of Expected
X1	0.171	0.171	
X1.5	0.138	0.114	121.1
X2.25	0.082	0.076	107.9

<Crossreactivity>

Related peptides	Crossreactivity (%)
CRF(1-41) (Mouse, Rat, Human)	100
CRF(17-41) (Mouse, Rat, Human)	0.1
ACTH (Human)	0.01
ACTH (Mouse, Rat)	0.01
Urocortin 1 (Human)	0.01
Urocortin 1 (Mouse, Rat)	0.01
Urocortin 2 (Mouse)	0
Urocortin 3 (Mouse, Rat)	0
PACAP27	0
PACAP38	0
VIP (Human, Porcine)	0

< Precision and reproducibility >

Test sample	Intra-assay CV (%)	Inter-assay CV (%)
Human plasma	1.17 – 4.34	1.46 – 9.15

<Assay range>

0.078 ~ 2.5 ng/mL

Ⅶ. Stability and Storage

< Storage >	Store all of the components at 2-8°C.
< Shelf life >	The kit is stable under the condition for 24 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

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