YK350 Active Ghrelin ELISA

FOR RESEARCH USE ONLY
NOT FOR DIAGNOSTIC USE

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Contents

Ⅰ.	**Introduction**	**2**
Ⅱ.	**Components**	**3**
Ⅲ.	**Method**	**3-6**
Ⅳ.	**Precaution**	**6-7**
Ⅴ.	**Stability and Storage**	**7**
Ⅵ.	**References**	**7**

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

YK350 Active Ghrelin ELISA

Ⅰ. Introduction

Ghrelin, a novel growth hormone releasing peptide is an acylated peptide that stimulates the release of growth hormone from pituitary. It was isolated from rat stomach and the structure was determined as a peptide consisting of 28-amino acid by Dr. Kenji kangawa (National Cardiovascular Center in Japan). The Ser3 residue of Ghrelin is modified by n-octanoic acid, a modification necessary for hormone activity.

This Active Ghrelin ELISA kit measures the active form of Ghrelin based on the principle of 2 Site Sandwich enzyme-linked immunosorbent assay (ELISA). It can detect not only octanoylated human Ghrelin but also octanoylated rat/mouse Ghrelin (1-28). This kit is manufactured using the high specific antibody pairs generated by Dr. Kangawa and by following his protocol. (patent pending : PCT WO 01/07475 A1)

YK350 Active Ghrelin ELISA Kit	**Contents**
▼ The assay kit can measure active ghrelin within the range of 2.9 ~ 184 fmol/mL	1) Monoclonal antibody against active ghrelin coated plate
▼ The assay is completed within 2hr+1hr+0.5hr.	2) Standard 3) HRP labeled monoclonal antibody against ghrelin solution
▼ With one assay kit, 40 samples can be measured in duplicate.	4) Substrate solution (TMB) 5) HRP dilution buffer
▼ Test sample: human/rat/mouse plasma Sample volume: 50 μL	6) Stopping solution 7) Assay buffer
▼ The 96-wells plate in kit is consisted by 8-wells strips, and the strips can be used separately.	8) Washing buffer (concentrated) 9) Transparent sheet
▼ Stability and storage Store all of the components at 2-8ºC. The kit is stable under the condition for 12 months from the date of manufacturing. The expiry date is stated on the label of kit.	

Ⅱ. Components

	Component	Form	Quantity	Main Ingredient
1.	Antibody coated plate	microtiter plate	1 plate (96 wells)	Mouse anti active ghrelin monoclonal antibody coated
2.	Standard	lyophilized	**1 vial (184 fmol)**	Synthetic human active ghrelin
3.	HRP labeled antibody solution	liquid	**1 tube (0.15 mL)**	HRP labeled mouse anti ghrelin monoclonal antibody
4.	Substrate solution	liquid	**2 bottles (11mLx2)**	3,3',5,5'-Tetramethylbenzidine (TMB)
5.	HRP dilution buffer	liquid	1 bottle (22 mL)	Phosphate buffer
6.	Stopping solution	liquid	1 bottle (6mL)	**1 mol/L H_2SO_4**
7.	Assay buffer	liquid	1 bottle (22 mL)	Buffer containing a reaction accelerator
8.	Washing buffer concentrate	liquid	1 bottle (40 mL)	Concentrated phosphate buffer
9.	Transparent sheet		3 pieces	

Ⅲ. Method

<Equipment & Reagent for requested>

1. Plate washer
2. Plate reader (450 nm measurement available)
3. Vortex mixer

<Preparation of sample>

Ghrelin is very unstable. Be careful to avoid any fragmentation or inactivation. All biological fluid should be treated with protease inhibitor such as aprotinin. It is also required to inhibit the esterase activity. Standard procedure of human blood sample preparation is described below.

Collect into the bleeding tubes which contain 500 KIU (Kallikrein Inhibitor Unit) of aprotinin and 1.25mg of EDTA-2Na per 1mL of whole blood. Rock the tubes gently and then immediately centrifuge

the blood sample (1500 ×g, 15 min at 4℃). Earned plasma should be immediately treated with 1/10 volume of 1 mol/L HCl. Sample must be kept below－40℃ for long term storage.

<Reagents preparation>

1. Dilute the washing buffer concentrate with ×20 volume of distilled water.
   Store the diluted washing buffer in refrigerator and use within 2 weeks.
2. Reconstitute the Standard (Lyophilized) with 1 mL of distilled water (→Standard #1). **Keep still approximately 10 minutes and vortex well.**
   Then dilute the standard as follows:

Standard No.	Std Vol.	Assay buffer
#2	#1 → 500 µL	500 µL
#3	#2 → 500 µL	500 µL
#4	#3 → 500 µL	500 µL
#5	#4 → 500 µL	500 µL
#6	#5 → 500 µL	500 µL
#7	#6 → 500 µL	500 µL

   The lyophilized standard contains 184 fmol of human active ghrelin.

   The dilution procedure described above is for 2.9 ～ 184 fmol/mL of the standard curve.

3. **Dilute only the required volume of the HRP labeled antibody solution with x200 volume of HRP dilution buffer and vortex well.**
   (→Prepare the diluted HRP labeled antibody solution more than one hour before using and use it within a day)

<Assay Procedure>

Pre-warm all reagents to room temperature prior to setting up the assay.
Do not dry up the wells during a measurement.

1. 150 µL of assay buffer is poured into the testing well. 50 µL of samples and standards are added into the each well. As a "Blank", 50 µL of assay buffer is added into the testing well. Then shake the plate gently. Manufacturer recommends to test duplicate for each sample. Plate is covered with transparent sheet and is incubated for 2 hours at RT.

2. Aspirate samples from wells and wash by washing buffer for 3 times. **Washing buffer volume: 350 µL**. Keep 1 min of interval before removing the washing buffer from wells. For removing the remnant completely, testing plate is tapped on a paper towel upside down. 200 µL of diluted HRP labeled antibody solution is poured into the wells. Testing plate is covered with transparent sheet and is incubated for 1 hour at RT.

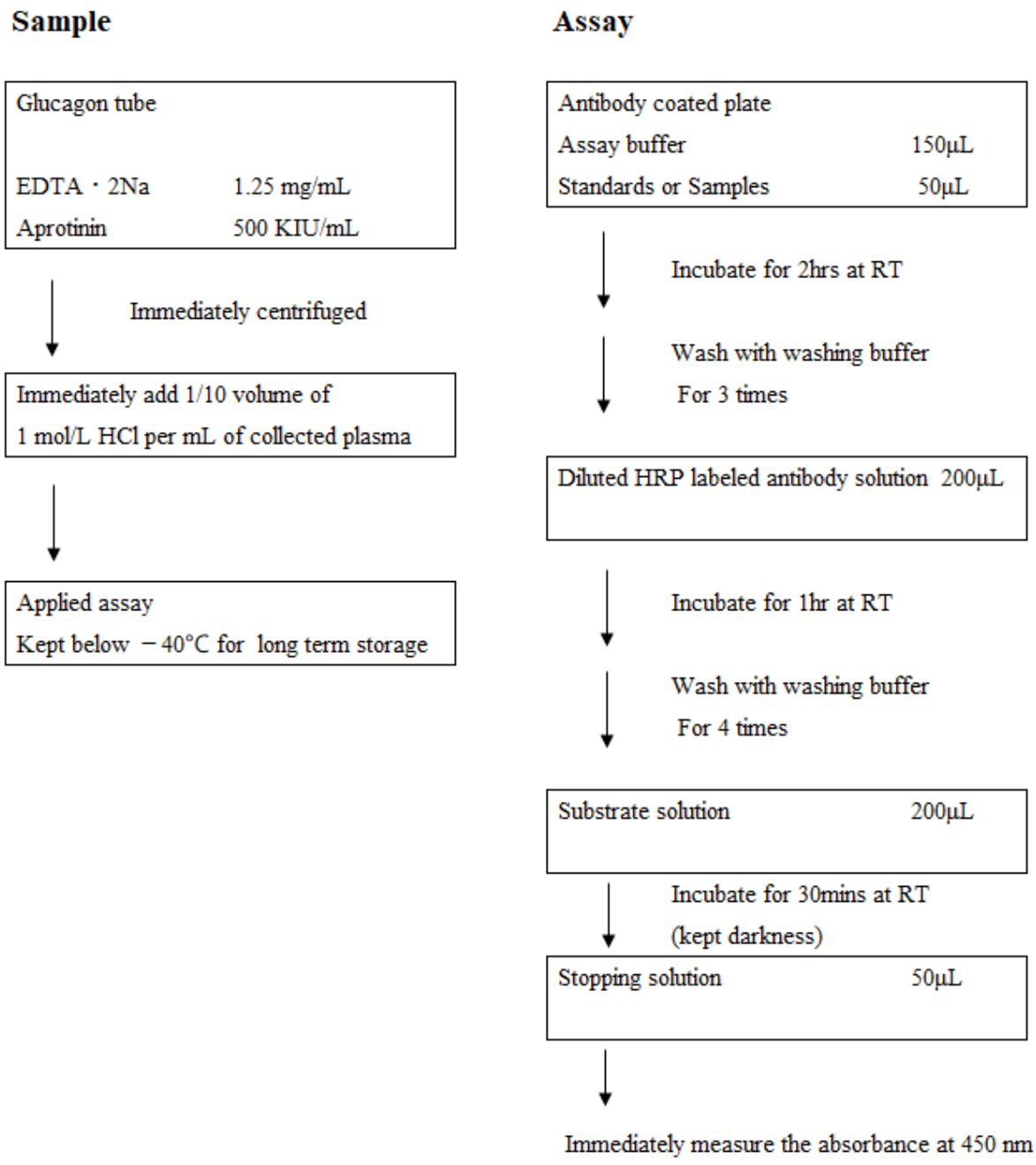
3. Aspirate samples from wells and wash by washing buffer for 4 times. **Washing buffer volume:**

350 µL. Keep 1 min of interval before aspirating the washing buffer from wells. For removing the remnant completely, testing plate is tapped on a paper towel upside down. 200 µL of substrate solution is poured into each well and is incubated for 30 min at RT with shading. After the incubation, 50 µL of stopping solution is added to each well to stop reaction. Then shake the plate gently.

4. Measure the absorbance of each well at 450 nm immediately.

5. The results of unknown samples can be calculated with any computer program having a 4 (or 5)-parameter logistic function. Otherwise plot the standard concentration (X-axis) and its corresponding absorbance (Y-axis). The concentration of active ghrelin in unknown sample is determined by plotting the sample's absorbance on the standard curve.
   When the HCl is added to the samples, multiply the results by 1.1 to offset the dilution.

Active Ghrelin ELISA Protocol



Ⅳ. Precaution

1. This kit is for research purpose only. Not for diagnostic use.

2. Warning-potential biohazardous material. Samples should be handled at the Biosafety level 2 as recommended for any potentially infectious human serum or blood samples in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories", 1984. In addition, handle and dispose of the Antibody coated plate as

well as all material coming into contact with them or with the ample as if capable of transmitting infection.

3. Substrate solution is sensitive to contamination from a variety of oxidizing agents such as bacteria, dust, metals and commonly used laboratory glassware. Avoid contacting with any potential source of these contaminations. Substrate solution is also sensitive to light. Avoid unnecessary exposure to light.

4. Stopping solution contains 1M sulfuric acid solution. Sulfuric acid is corrosive and can cause eye and skin burns. Avoid contact with skin and eyes. To prevent any contact, wear protective equipment such as safety gloves, rubber gloves, as appropriate.

5. Reagents are stored between +2ºC and +8ºC. Before the measurement, all reagents must be equilibrated to room temperature. Put unused strips back in the aluminum pouch immediately, because strips are affected by humidity.

6. Do not mix the reagents from different kits unless they have the same lot numbers.

7. Do not use reagents after the expiration date printed on the label.

8. Occasionally, the assay buffer and the washing buffer concentrate generate some precipitates. However, they can be resolved by raising the temperature from room temperature to approximately 30ºC. After then, you can use their buffers.

9. Dilute the high level samples with the assay buffer

Ⅴ. Stability and Storage

< Storage >	Store all of the components at 2-8°C.
< Shelf life >	The kit is stable under the condition for 12 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

Ⅵ. References

1. Kojima, M. et al.: Nature, 402: 656, 1999
2. Inui, A.: Nat. Rev. Neurosci., 2: 551, 2001
3. Date, Y. et al. : Endocrinology, 141 : 4255, 2000
4. Gualillo, O. et al. : Endocrinology, 142: 788, 2001
5. Matsumoto, M. et al.: Biochem. Biophys. Res. Commun., 287: 142, 2001
6. Hosoda, H. et al.: Biochem. Biophys. Res. Commun., 279: 909, 2000

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Create at March 10, 2023