DATA SHEET

Jan.,2004 **97751**

Active Ghrelin ELISA Kit

US patent No.) US7385026B1

European Patent No.) EP1197496B1

Read this instruction before use.

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 Ghreline ELISA kit measures the active form of Ghreline based on the principle of 2 Site Sandwich enzyme-linked immunosorbent assay(ELISA). It can detect not only octanoylated human Ghreline but also octanoylated rat/mouse Ghreline(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)

CAUTION:

For research use only. Not for diagnostic use.

Components:

1.	Standard (Lyophilized)	1	vial
	*The exact value of lyophilized standard is showed in analysis sheet.		
2.	Assay buffer	22	mL
3.	Antibody coated plate	8x12	wells
4.	HRP conjugated antibody	250	μL
5.	HRP dilution buffer	22	mL
6.	Substrate solution	22	mL
7.	Stop reagent (0.5 mol/L H_2SO_4)	6	mL
8.	Washing buffer concentrate	40	mL

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 xg, 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 x20 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). 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 approximately 160 fmol of human active ghrelin. (The exact value of lyophilized standard is showed in analysis sheet.)
   The dilution procedure described above is for about 2.5 ～ 160 fmol/mL of the standard curve.
3. Dilute only the required volume of the HRP conjugated antibody with x100 volume of HRP dilution buffer.
   (→diluted HRP conjugated antibody)

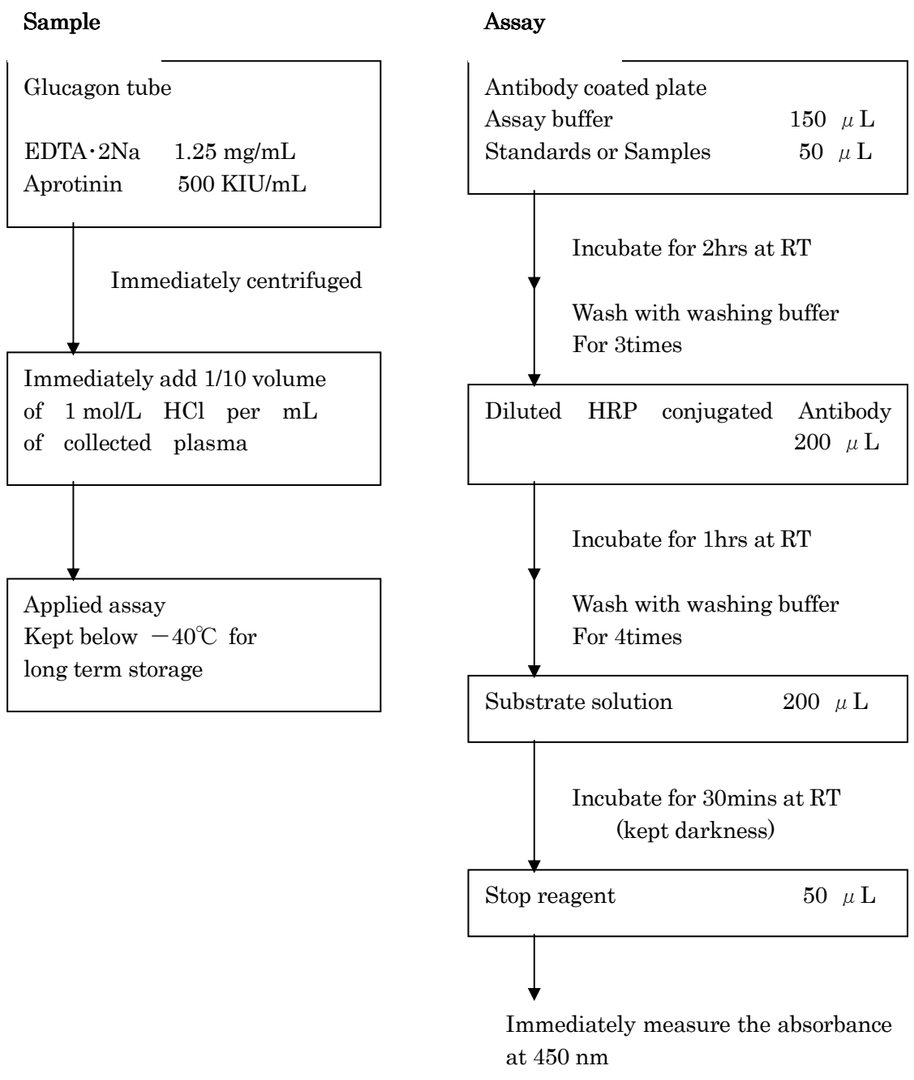
Assay Procedure:

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 samples. 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: 300 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 conjugated antibody 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: 300 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 wells and is incubated for 30 min at RT with shading. After the incubation, 50 μL of stop reagent 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. 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



PRECAUTION

1. This kit is for research purpose only. Not for diagnostic use.
2. Warning-potential biohazardous material. Specimens should be handled at handled at the Biosafety level 2 as recommended for any potentially infectious human serum or blood specimen 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 specimens 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. Stop solution contains 0.5M 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 equipments such as safety gloves, rubber gloves, as appropriate.
5. Reagents are stored between +2℃ and +8℃. Before the measurement, all reagents must be equilibrated to room temperature.
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.

REFERENCE

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