

## Desacyl-Ghrelin ELISA Kit

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 Desacyl-Ghreline ELISA kit measures only the Desacyl-Ghreline based on the principle of 2 Site Sandwich enzyme-linked immunosorbent assay(ELISA). It can detect not only human Desacyl-Ghreline but also rat/mouse Desacyl-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 Analytical Data sheet. |              |
| 2. Assay buffer   | 22 mL        |
| 3. Antibody coated plate  | 8 x 12 wells |
| 4. HRP conjugated antibody  | 250 $\mu$ L  |
| 5. HRP dilution buffer  | 22 mL        |
| 6. Substrate solution   | 22 mL        |
| 7. Stop reagent ( 0.5 mol/L H <sub>2</sub> SO <sub>4</sub> )                | 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. Some extraction methods should be useful in many cases. 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  $\times$ g, 15 min at 4  $^{\circ}$ C ).

Acidified plasma with 1/10 volume of 1N HCl ( prepared for Active ghrelin measurement ) is available for this assay.

Sample must be kept below  $-40^{\circ}$ C for long term storage.

### Reagents preparation:

1. Dilute the washing buffer concentrate with  $\times 20$  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 (  $\rightarrow$ Standard #1 ).  
Then dilute the standard as follows:

Standard No.	Std Vol.	Assay buffer
#2	#1 $\rightarrow$ 500 $\mu$ L	500 $\mu$ L
#3	#2 $\rightarrow$ 500 $\mu$ L	500 $\mu$ L
#4	#3 $\rightarrow$ 500 $\mu$ L	500 $\mu$ L
#5	#4 $\rightarrow$ 500 $\mu$ L	500 $\mu$ L
#6	#5 $\rightarrow$ 500 $\mu$ L	500 $\mu$ L
#7	#6 $\rightarrow$ 500 $\mu$ L	500 $\mu$ L

The lyophilized standard contains approximately 800 fmol of human desacyl-ghrelin. ( The exact value of lyophilized standard is showed in Analytical Data sheet. )

The dilution procedure described above is for about 12.5  $\sim$  800 fmol/mL of the standard curve.

3. Dilute only the required volume of the HRP conjugated antibody with x100 volume of HRP dilution buffer.  
(  $\rightarrow$ Prepare the diluted HRP conjugated antibody 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.

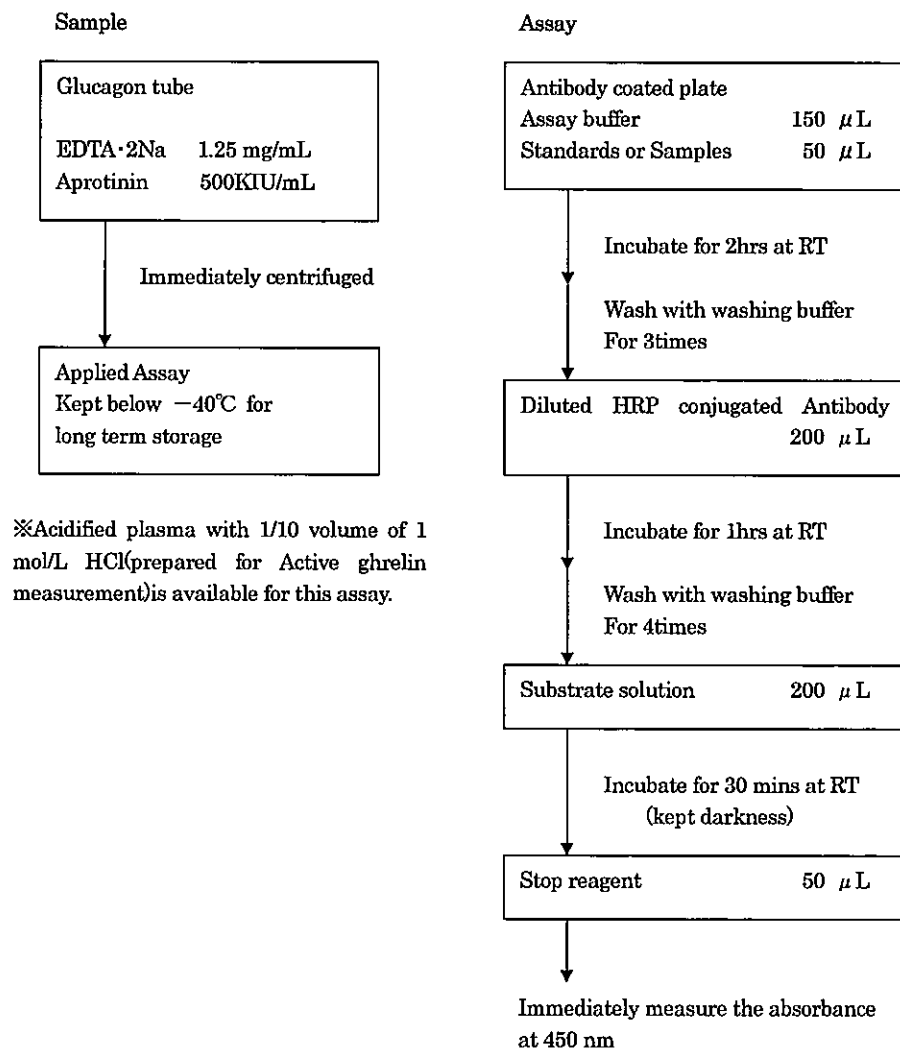
Do not dry up the wells during a measurement.

1. 150  $\mu$  L of assay buffer is poured into the testing well. 50  $\mu$  L of samples and standards are added into the each well. As a "Blank", 50  $\mu$  L 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: 400  $\mu$  L. Keep 1 min of interval time before removing the washing buffer from wells. For removing the remnant completely, testing plate is tapped on a paper towel upside down. 200  $\mu$  L 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: 400  $\mu$  L. Keep 1 min of interval time before aspirating the washing buffer from wells. For removing the remnant completely, testing plate is tapped on a paper towel upside down. 200  $\mu$  L of substrate solution is poured into each wells and is incubated for 30 min at RT with shading. After the incubation, 50  $\mu$  L of stop reagent is added to each well to stop reaction. Then shake the plate gently.

4. Measure the absorbance of each wells at 450 nm immediately.
5. Plot the standard concentration (X-axis) and its corresponding absorbance (Y-axis). The concentration of desacyl-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.

### Desacyl-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 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°C and +8°C. 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 30°C. After then, you can use their buffers.
9. Dilute the high level specimens with the assay buffer.

### STORAGE

2-8°C

### REFERENCE

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Distributor : SCETI K.K.

Kasumigaseki Place, 3-6-7 Kasumigaseki,  
Chiyoda-ku, Tokyo 100-0013 JAPAN

TEL: +81-3-5510-2652

FAX: +81-3-5510-0133

<http://www.sceti.co.jp/english/export/>

LSI Medience Corporation

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