

YK351 Desacyl-Ghrelin ELISA

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NOT FOR DIAGNOSTIC USE**

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

YK351 Desacyl-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 Desacyl-Ghrelin ELISA kit measures only the Desacyl-Ghrelin based on the principle of 2 Site Sandwich enzyme-linked immunosorbent assay (ELISA). It can detect not only human Desacyl-Ghrelin but also rat/mouse Desacyl-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)

YK351 Desacyl-Ghrelin ELISA Kit	Contents
▼ The assay kit can measure desacyl-ghrelin within the range of 13.6~ 870 fmol/mL	1) Monoclonal antibody against desacyl-ghrelin coated plate
▼ The assay is completed within 2hr+1hr+0.5hr.	2) Standard
▼ With one assay kit, 40 samples can be measured in duplicate.	3) HRP labeled monoclonal antibody against ghrelin solution
▼ Test sample: human/rat/mouse plasma Sample volume: 50 µL	4) Substrate solution (TMB)
▼ The 96-wells plate in kit is consisted by 8-wells strips, and the strips can be used separately.	5) HRP dilution buffer
▼ Stability and storage Store all of the components at 2-8°C. 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.	6) Stopping solution
	7) Assay buffer
	8) Washing buffer (concentrated)
	9) Transparent sheet

II. Components

Component	Form	Quantity	Main Ingredient
1. Antibody coated plate	microtiter plate	1 plate (96 wells)	Mouse anti desacyl-ghrelin monoclonal antibody coated
2. Standard	lyophilized	1 vial (870 fmol)	Synthetic human desacyl-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 H₂SO₄
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. 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 ×g, 15 min at 4°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°C 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 870 fmol of human desacyl-ghrelin.

The dilution procedure described above is for 13.6 ~ 870 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. 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 μ L.** 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 μ L of substrate solution is poured into each well and is incubated for 30 min at RT with shading. After the incubation, 50 μ L 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 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

Sample

Glucagon tube	
EDTA · 2Na	1.25mg/mL
Aprotinin	500 KIU/mL

↓
Immediately centrifuged

Applied Assay Kept below -40°C for long term storage

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

Assay

Antibody coated plate	
Assay buffer	150μL
Standards or Samples	50μL

↓
Incubate for 2hrs at RT
↓
Wash with washing buffer
For 3 times

Diluted HRP labeled antibody solution 200μL

↓
Incubate for 1hr at RT
↓
Wash with washing buffer
For 4 times

Substrate solution	200μL
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↓
Incubate for 30mins at RT
(kept darkness)

Stopping solution	50μL
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↓
Immediately measure the absorbance at 450nm

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 +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 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

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2. Inui, A.: Nat. Rev. Neurosci., 2: 551, 2001
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5. Matsumoto, M. et al.: Biochem. Biophys. Res. Commun., 287: 142, 2001
6. Hosoda, H. et al.: Biochem. Biophys. Res. Commun., 279: 909, 2000

<Manufacturer>

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