



**KAMIYA BIOMEDICAL COMPANY**

# Human, Mouse and Rat Ghrelin ELISA, Active

**For the quantitative determination of  
Active Ghrelin in human, mouse and rat plasma.**

**Cat. No. KT-364**

**For Research Use Only.**

**PRODUCT INFORMATION****Human, Mouse and Rat Ghrelin ELISA, Active**  
**Cat. No. KT-364****INTENDED USE**

The Human, Mouse and Rat Ghrelin ELISA, Active is for the quantitative determination of active Ghrelin in human, mouse and rat plasma. For research use only. Not for use in diagnostics procedures.

**INTRODUCTION**

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

**PRINCIPLE**

This Active Ghrelin ELISA kit measures the active form of Ghrelin based on the principle of a 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 highly specific antibody pairs.

**COMPONENTS**

<u>Component</u>	<u>Form</u>	<u>Quantity</u>
1. Ghrelin Calibrator	Lyophilized	1 vial
2. Assay Buffer	Liquid	1 vial (22 mL)
3. Antibody-Coated Plate		8 x 12 wells
4. HRP-Conjugated Ab Concentrate	Liquid	1 vial (250 µL)
5. HRP Dilution Buffer	Liquid	1 bottle (22 mL)
6. Substrate Solution	Liquid	1 bottle (22 mL)
7. Stop Solution (0.5 M H <sub>2</sub> SO <sub>4</sub> )	Liquid	1 bottle (6 mL)
8. Wash Buffer Concentrate	Liquid	1 bottle (40 mL)
9. Plate Seals		3

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Plate washer
- Plate reader (450 nm measurement available)
- Vortex mixer

**STORAGE**

Store kit at 4°C.

**SPECIMEN COLLECTION AND HANDLING**

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

Collect blood into tubes containing 500 KIU (Kallikrein Inhibitor Unit) of aprotinin and 1.25 mg of EDTA-2Na per 1 mL of whole blood. Rock the tubes gently, then immediately centrifuge the blood sample (1,500 x g, 15 min. at 4°C). The prepared plasma should be immediately treated with 1/10 volume of 1 M HCl. Samples must be kept below -40°C for long term storage.

## REAGENT PREPARATION:

1. Dilute the Wash Buffer Concentrate 20X with distilled water. Store the diluted Wash Buffer at 4°C and use within 2 weeks.
2. Reconstitute the Ghrelin Calibrator (Lyophilized) with 1 mL of distilled water (Cal #1). Then dilute the reconstituted Calibrator as follows:
 

Cal No.	Cal. Vol.	Assay buffer
#2	500 µL of Cal #1	500 µL
#3	500 µL of Cal #2	500 µL
#4	500 µL of Cal #3	500 µL
#5	500 µL of Cal #4	500 µL
#6	500 µL of Cal #5	500 µL
#7	500 µL of Cal #6	500 µL

The lyophilized Ghrelin Calibrator contains approximately 160 fmol of human active Ghrelin (The exact value of lyophilized calibrator is showed on the vial label.). The dilution procedure described produces a calibrator range of approximately 2.5 - 160 fmol/mL.
3. Dilute only the required volume of the HRP-Conjugated Antibody with 100X volume of HRP Dilution Buffer (diluted HRP-conjugated antibody).

## ASSAY PROTOCOL

Warm the reagents to room temperature before beginning the test.

Do not allow the wells to dry during the assay.

1. Add 150 µL of Assay Buffer into wells. 50 µL of samples and prepared Calibrators are added into the appropriate well. As a "Blank", 50 µL of Assay Buffer is used. Then shake the plate gently. We recommend duplicate testing for each sample. Cover the plate with a Plate Seal and incubate for 2 hours at RT.
2. Aspirate samples from wells and wash 3 times with 300 µL of diluted Washing Buffer. Wait for an interval of 1 minute before removing the Washing Buffer from wells. To remove the remnant completely, the plate is tapped upside down on a paper towel. Add 200 µL of diluted HRP-Conjugated Antibody into the wells. The plate is covered with a Plate Seal and is incubated for 1 hour at RT.
3. Aspirate solution from the wells and wash 4 times with 300 µL of diluted Wash Buffer. Wait for an interval of 1 minute before aspirating the Wash Buffer from the wells. To remove the remnant completely, the plate is tapped upside down on a paper towel. Add 200 µL of Substrate Solution into each well and incubate for 30 minutes at RT, protected from light. After incubation, add 50 µL of Stop Solution to each well to stop the reaction. Then shake the plate gently.
4. Immediately measure the absorbance of each well at 450 nm.

## RESULTS

Plot the calibrator concentration ( X-axis ) and its corresponding absorbance ( Y-axis ). The concentration of active Ghrelin in unknown samples is determined by plotting the sample's absorbance on the calibration curve. When the HCl is added to the samples, multiply the results by 1.1 to offset the dilution.

## PRECAUTIONS

1. This kit is for research use only. Not for use in diagnostic procedures.
2. Warning: Potentially biohazardous material. Specimens should be handled at the Biosafety level 2 as recommended for any potentially infectious human serum or blood specimen according to 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 it 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 contact with any potential source of these contaminations. Substrate Solution is sensitive to light. Avoid unnecessary exposure to light.
4. Stop Solution contains 0.5 M 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 and rubber gloves as appropriate.
5. Reagents are stored at 4°C. Before testing, 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.
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.

## PROTOCOL SUMMARY

### Sample

Ghreltin Tube	
EDTA • 2Na	1.25 mg/mL
Aprotinin	500 KIU/mL

↓ Centrifuge Immediately

Immediately add 1/10 volume of 1 M HCL per mL of collected plasma.

↓

Use prepared sample in assay.  
Store long term at -40°C or below.

### Assay

Antibody-Coated Plate	
Assay Buffer	150 µL
Calibrators or Samples	50 µL

↓ Incubate for 2 hours at RT

↓ Wash 3x with Wash Buffer

Diluted HRP-Conjugated Ab	
	200 µL

↓ Incubate for 1 hour at RT

↓ Wash 4x with Wash Buffer

Substrate Solution	
	200 µL

↓ Incubate for 30 min. at RT  
(protect from light)

Stop Solution	
	50 µL

↓ Immediately measure the absorbance at 450 nm

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