PRO-MOTILIN (HUMAN) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN PRO MOTILIN CONCENTRATIONS IN PLASMA AND SERUM

ALWAYS REFER TO LOT SPECIFIC PROTOCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ AND CHECK ALL ITEMS OF EACH KIT BEFORE USING THIS PRODUCT.

FOR RESEARCH USE ONLY.NOT FOR USE IN DIAGNOSTIC PROCEDURES.

PRODUCT INFORMATION:

THIS IS FOR ONE TIME USE ONLY.

ELISA NAME	**PRO MOTILIN (HUMAN) ELISA KIT**
Catalog No.	SK00214-06
Lot No.:	
Formulation	96 T
Standard range	0.2 – 12.8 ng/mL
Sensitivity	0.1 ng/mL
Sample Volume	100 µL
Dilution Factor	**Optimal dilutions should be determined by each laboratory for each application**
Sample Type	Serum, Plasma,
Specificity	Human
Calibration	Human Pro Motilin (26-115) (HEK293)
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8 °C for 8 months, see page 2-3 for detail
This kit contains sufficient materials to run 35~40 samples duplicated provided that assay is run according to protocol.	

THIS IS ONE TIME USE ONLY

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DESCRIPTION

This Pro Motilin (Human) ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human Pro Motilin from serum and plasma in a sandwich ELISA format.
This immunoassay contains recombinant human Pro Motilin and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural Pro Motilin samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with an antibody specific for human Pro Motilin. The capture antibody can bind to the human Pro Motilin in the standard and samples. After washing the plate of any unbound substances, a biotinylated antibody against human Pro Motilin is added to the wells. After another washing of the plate, the Streptavidin-HRP Conjugate is added. After the last wash to remove any unbound enzyme, a substrate solution (TMB) is added to the wells and color develops in direct proportion to the amount of Pro Motilin bound in the standard solutions or samples. A standard curve can be established and sample values can be read off the standard curve.

PROCEDURAL LIMITATIONS

_FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
_This ELISA kit should not be used beyond the expiration date on the kit label.
_Do not mix reagents with those from other lots or sources.
_It is important that the Dilution Buffer selected for the standard curve be consistent with the samples being assayed.
_Each laboratory must determine the optimal dilution factors for the samples being assayed with a pretest. If samples generate values that are not within the dynamic range of the standard curve, further concentrate or dilute the samples as required with Dilution Buffer and repeat the assay.
_Any modifications in buffers, pipetting technique, washing technique, incubation time or temperature, as well as kit age can cause a change in signal.
_Not all interfering factors have been tested in the immunoassay, therefore the possibility of interference cannot be excluded.

COMPONENTS PROVIDED

DESCRIPTION	CODE	QUANTITY
Pro Motilin Microplate – 96 well microplate coated with antibody specific for human Pro Motilin.	**214-06-01**	**1 plate**
Pro-Motilin Standard – refer to lot of lyophilized recombinant human Pro Motilin.	**214-06-02**	**1 vial**
Detection Antibody Concentrate – refer to lot 10-fold concentrate of lyophilized biotinylated antibody against human Pro Motilin.	**214-06-03**	**1 vial**
Positive Control – one vial of lyophilized recombinant human Pro Motilin	**214-06-04**	**1 vial**
Streptavidin-HRP Conjugate – 120 µL/vial of 100-fold concentrated solution of Streptavidin-HRP conjugate.	**SAHRP**	**1 vial**
Dilution Buffer – 45 mL of buffered solution with preservative.	**DB01**	**1 bottle**
HRP Diluent Solution – 12 mL of buffered solution with preservative.	**DB08C**	**1 bottle**
Wash Buffer – 50 mL of 10-fold concentrated buffered surfactant with preservative.	**WB01**	**1 bottle**
TMB Substrate Solution – 11 mL of TMB substrate solution.	**TMB01**	**1 bottle**
Stop Solution – 11 mL of 0.125M HCl.	**S-STOP**	**1 bottle**
Plate Sealer	**EAPS**	**1 piece**
Plastic Pouch	**P01**	**1 piece**

STORAGE

Unopened Kit: Store at 2 – 8 °C for up to 8 months. For longer storage for up to 12 months, unopened Standard, Positive Control, Detection Antibody Concentrate, Dilution Buffer and HRP Diluent Solution should be stored at -20 ~ -70 °C.

Streptavidin-HRP Conjugate 100-fold concentrated solution and TMB Substrate Solution should be stored only at 2 – 8 °C (**DO NOT FREEZE** and **PROTECT FROM LIGHT**). Do not use kit past expiration date.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (350 – 400 rpm).
- Microplate washer or manifold dispenser.
- 100 mL and 500 mL graduated cylinders.
- Multi-channel Pipette, Pipettes and pipette tips.
- Deionized or distilled water.

PRECAUTION

This kit should be handled by those persons who have been trained in and can follow the principles of good laboratory practice. Wear protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken while handling solutions in this kit to avoid contact with skin or eyes, especially with the stop solution because it contains diluted hydrochloric acid. Wash immediately with water in case of contact on skin or eyes.

SAMPLE COLLECTION AND STORAGE

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤-20°C. Avoid repeated freeze-thaw cycles.

Cell Culture- All samples should be used animal free media. The fetal bovine serum or other animal serum may cross-react and or interface with this elisa assay.

Note: Use Aprotinin (enzyme inhibitor) (Code No.: 00700-01-25) for ALL sample collection to prevent sample degradation. 0.5 TIU per ml of sample solution.

SAMPLE PREPARATION

Optimal dilutions should be determined by each laboratory for each application with a pretest.

Use polypropylene test tubes.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Wash Buffer – Dilute 50 mL of Wash Buffer Concentrate into 450 mL distilled or deionized water to make 500 mL of 1x Wash Buffer. If crystals have formed in the concentrate, warm bottle in a water bath until the crystals have completely dissolved.

Pro-Motilin Standard – Reconstitute the human Pro-Motilin standard with refer to lot of Dilution Buffer. Allow the stock standard to sit for at least 15 minutes with gentle agitation until completely dissolved prior to making standard dilutions (see below). Mix each tube thoroughly before the next transfer.

TUBE	STANDARD	DILUTION BUFFER	CONCENTRATION
Stock	powder	Refer to lot	XXXX
# 1	Refer to lot	Refer to lot	12.8 ng/mL
# 2	150µL of 1	450 µL	3.2 ng/mL
# 3	150µL of 2	450 µL	0.8 ng/mL
# 4	150µL of 3	450 µL	0.2 ng/mL

Detection Antibody - Reconstitute the Detection Antibody Concentrate with refer to lot of Dilution Buffer to produce a 10-fold concentrated stock solution. Allow the concentrated solution to sit for at least 5 minutes until completely dissolved. Pipette 9.45 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 1.05 mL of 10-fold concentrated stock solution to prepare working solution.

Streptavidin-HRP Conjugate - Pipette 11.88 mL of HRP Diluent Solution into a 15 mL centrifuge tube and transfer 120 µL of 100-fold concentrated stock solution to prepare working solution. **Note: (protect from light)**. **DO NOT FREEZE.** The working solution of Streptavidin-HRP Conjugate should be freshly prepared and used within a few hours.

Positive Control - Reconstitute the Positive Control refer to lot.

ELISA PROTOCOL

Bring all reagents and samples to room temperature before the start of the assay. Blank, standard dilutions, positive control and samples should be assayed in duplicate. ELISA Protocol may need further optimization.

1. Prepare all reagents, standard dilutions, positive control and samples as directed previously.
2. Remove unneeded microplate strips from the plate frame and return them to the plastic pouch with the desiccant pack.
3. Add 100 µL per well of **Dilution Buffer** to Blank wells.
4. Add 100 µL per well of **Standard Dilutions** in reverse order of serial dilution, **sample,** or **positive control** . Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature. (Please see plate layout provided.)
5. Aspirate and wash each well with 300 µL of **1x Wash Buffer** four times. After the last wash, aspirate any remaining 1x Wash Buffer, invert the plate and blot against clean paper towel(s).
6. Add 100 µL per well of **Detection Antibody working solution**. Cover with plate sealer and incubate for 90 min on microplate shaker at room temperature.
7. Repeat the aspiration and wash as in step 5.
8. Add 100 µL per well of working solution of **Streptavidin HRP conjugate**. Cover with plate sealer and incubate for 45 minutes on microplate shaker at room temperature. **Protect from light.**
9. Repeat the aspiration and wash as in step 5.
10. Add 100 µL per well of **Substrate Solution**. Incubate for refer to lot on microplate shaker at room temperature. **Protect from light.**
11. Add 100 µL per well of **Stop Solution**. The color in the wells should change from blue to yellow. If the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
12. Read plate using a microplate reader set to 450 nm within 5 minutes.

CALCULATION OF RESULTS

Create a standard curve by plotting the log of the known concentrations of the standard dilutions (x-axis) versus the log of its corresponding O.D. (y-axis) and draw the best fit line through the points. It is recommended to use computer software capable of generating a log-log or 4-parameter curve fit to more accurately quantify the standard dilutions.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

SPECIFICITY

PROTEIN	CROSS-REACTIVITY
Human Pro Motilin (HEK293)	100%
Human Ghrelin	0
Human GIP	0
Human Gastrin	0

TYPICAL STANDARD CURVE

This standard curve is for demonstration only. A new standard curve should be made for each set of samples assayed.

STANDARD (NG/ML)	AVERAGE OD450 NM (CORRECTED)
Blank	0 (0.099)
0.2	0.040
0.8	0.153
3.2	0.617
12.8	2.394

SUMMARY OF ASSAY PROCEDURE

PREPARE REAGENTS, SAMPLES AND STANDARD DILUTIONS
↓
Add 100 µL of standard dilutions, samples and positive control. Cover with plate sealer and incubate 2 hours on microplate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Detection Antibody working solution. Cover with plate sealer and incubate 90 min on microplate shaker at RT.
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Streptavidin-HRP Conjugate working solution. Cover with plate sealer and incubate 45 minutes on microplate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Substrate Solution. Incubate refer to lot on microplate shaker at RT. **Protect from light.**
↓
Add 100 µL per well of Stop Solution. Read at 450 nm within 5 minutes.