HUMAN RECEPTOR TYROSINE-PROTEIN KINASE ERBB-3 (ErbB3)/ TYROSINE KINASE-TYPE CELL SURFACE RECEPTOR HER3 (Her3) ELISA KIT

FOR THE QUANTITATIVE DETERMINATION OF HUMAN ERBB3/HER3 CONCENTRATIONS IN SERUM AND PLASMA.

ALWAYS REFER TO LOT SPECIFIC PROTCOL PROVIDED WITH EACH KIT FOR INSTRUCTIONS. PROTOCOL MUST BE READ BEFORE USING THIS PRODUCT.

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

PRODUCT INFORMATION:

ELISA NAME	HUMAN ERBB3/HER3 ELISA
Catalog No.	SK00469-07
Formulation	96 T
Lot No.	
Standard range	23.44-1500 pg/mL
Sensitivity	10 pg/mL
Sample Volume	100 µL
Dilution Factor	Optimal dilutions should be determined by each laboratory for each application
Sample Type	Serum, plasma
Specificity	Human Her3
Intra-assay Precision	4 - 6%
Inter-assay Precision	8 - 10%
Storage	2 – 8 °C
This kit contains sufficient materials to run 35 samples duplicated provided that assay is run according to protocol.	

Order Contact:
AVISCERA BIOSCIENCE, INC.
2348 Walsh Ave., Suite C
Santa Clara, CA 95051
USA
Tel: (408) 982 0300
Fax: (408) 982 0301
Email: Sales@AvisceraBioscience.com
Website: www.AvisceraBioscience.com

DESCRIPTION

This Human Soluble ErbB3/Her3 ELISA Kit contains the necessary components required for the quantitative measurement of recombinant and/or natural human soluble Her3 from serum and in a sandwich ELISA format.

This immunoassay contains recombinant human HER3and antibodies raised against this protein. Results from this immunoassay have shown to accurately quantify recombinant and natural human Her3 samples.

ASSAY OVERVIEW

This assay employs the quantitative sandwich ELISA format. The plate is pre-coated with a monoclonal antibody specific for human Her3. The capture antibody can bind to the mouse in the standard and samples. After washing the plate of any unbound substances, a monoclonal antibody HRP conjugated against human Her3 is added to the wells. 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 human Her3 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
Her3 Microplate – 96 well microplate coated with a monoclonal antibody specific for human Her3 .	**469-07-01**	**1 plate**
Human Her3 Standard – 1500 pg/vial of lyophilized recombinant human MMP-8.	**469-07-02**	**1 vial**
Detection Antibody Concentrate – 105µL/vial of 100-fold concentrate of the monoclonal antibody HRP conjugate against human Her3	**469-07-03**	**1 vial**
Positive Control – one vial of lyophilized recombinant human Her3	**469-07-04**	**1 vial**
Dilution Buffer – 60 mL of buffered solution with preservative.	**DB10**	**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 0.5M 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, unopened Standard, Positive Control and Detection Antibody Concentrate should be stored at -20 °C or -70 °C. Do not use kit past expiration date.
Opened / Reconstituted Reagents: Reconstituted Standard (stock) solution and Detection Antibody concentrated solution SHOULD BE STORED at -20 °C or -70 °C for up to one month. The anti mouse fetuin a IgG-HRP Conjugate 100-fold concentrated solution and TMB Substrate Solution can be stored at 2 – 8 °C for up to 8 months (**DO NOT FREEZE** and **PROTECT FROM LIGHT**). All other components may be stored at 2 – 8 °C for up to 8 months.
Microplate Wells: Return unused strips to the plastic pouch with the desiccant pack. Microplate may be stored for up to 6 months at 2 – 8 °C after opening.

ADDITIONAL MATERIALS REQUIRED

- Microplate reader capable of absorbance measurement at 450 nm.
- Microplate shaker (250 – 300 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). Allow blood to clot for 30 minutes. Centrifuge at 1000 x g for 15 minutes and collect serum. Assay samples immediately or aliquot and store 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.

Optional: Use Aprotinin (enzyme inhibitor) for ALL sample collection to prevent sample degradation. 0.5 TIU per mL of sample solution.

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.

Human HER3 Standard – Reconstitute the human HER3standard with 1.0 mL of Dilution Buffer. The concentration of the reconstituted stock solution is 1500 pg/mL. 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	1.0 mL	1500 pg/mL
# 1	250µL of stock	250µL	750 pg/mL
# 2	250µL of 1	250µL	375 pg/mL
# 3	250µL of 2	250µL	187.5 pg/mL
# 4	250µL of 3	250µL	93.75 pg/mL
# 5	250µL of 4	250µL	46.88 pg/mL
# 6	250µL of 5	250µL	23.44 pg/mL

Detection Antibody HRP - Pipette 9.395 mL of Dilution Buffer into a 15 mL centrifuge tube and transfer 0.105 mL of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution should be used within a few days **(protect from light)**. **DO NOT FREEZE.**

Positive Control - Reconstitute the Positive Control with 1.0 mL Dilution Buffer. **Note:** Positive Control could be used within a few days if stored at -20 °C or -70 °C.

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 (A2, A3).
4. Add 100 µL per well of **Standard Dilutions** in reverse order of serial dilution (G2, G3 to B2,B3), **sample,** or **positive control** (B4, B5). Cover with plate sealer and incubate for 2 hours on microplate shaker at room temperature.
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 2 hours on microplate shaker at room temperature. **Protect from light.**
7. Repeat the aspiration and wash as in step 5.
8. Add 100 µL per well of **Substrate Solution**. Incubate for 3-8 minutes 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 15 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 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	CROSSREACTIVITY(%)
HUMAN SOLUBLE HER3	**100**
HUMAN SOLUBLLE HER2	**0**
HUMAN SOLUBLE HER1	**0**

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

(PG/ML)	CORRECTED (450NM)
Blank	0 (0.099)
23.44	0.039
46.88	0.082
93.75	0.164
187.5	0.293
375	0.625
750	1.238
1500	2.117

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 HRP working solution. Cover with plate sealer and incubate 2 hours on microplate shaker at RT. **Protect from light.**
↓
Aspirate and wash 4 times.
↓
Add 100 µL per well of Substrate Solution. Incubate 3-8 min on microplate shaker at RT. **Protect from light.**
↓
Add 100 µL per well of Stop Solution. Read at 450 nm within 15 minutes.

TYPICAL STANDARD CURVE