EAGLE BIOSCIENCES

Humanized Anti-HER-2 (Herceptin/Trastuzumab) ELISA Assay Kit

Catalog Number:
AHR31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 1.1 (06.28.18)

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I. INTENDED USE

Eagle Biosciences Humanized Anti-HER-2 (Herceptin/Trastuzumab) ELISA Assay kit is intended for use in the quantitative determination of Herceptin levels in human serum and EDTA plasma. Eagle Biosciences Humanized Anti-HER-2 (Herceptin/Trastuzumab) ELISA Assay kit is intended for research use only.

II. INTRODUCTION

Herceptin (Trasuzumab) is a humanized monoclonal antibody used in cancer treatment for Human Epidermal Growth Factor Receptor 2 (HER2) over-expressing tumors. In the case of these tumors, Herceptin binds to and inhibits tumor growth. Herceptin can also be labeled with various toxins to enhance tumor suppression. Studies have also found that Herceptin targets gastric cancer stem cells characterized by CD90 phenotype.

III. ASSAY PRINCIPLE

Eagle Biosciences Humanized Anti-HER-2 (Herceptin/Trastuzumab) ELISA Assay kit utilizes the "sandwich" technique with HER2 coated to solid phase microtiter plate wells and an antibody to human IgG which is labeled with horseradish peroxidase used for detection. Assay calibrators, controls and diluted patient samples are added directly to wells of a micro titer plate that is coated with HER2 recombinant protein. Subsequently, horseradish peroxidase (HRP) conjugated human IgG antibody is added to each well. After the first incubation period, a "sandwich" of "HER2 - Herceptin – HRP conjugated human IgG antibody" is formed on the surface of the plate wells. The unbound proteins are removed in the subsequent washing step. For the detection of this immunocomplex, the wells are then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to the wall of each micro titer well is directly proportional to the amount of Herceptin in the calibrators. A calibration curve is generated by plotting the absorbance versus the respective Herceptin concentration for each calibrator using point-to-point or 4 parameter curve fitting. The concentration of Herceptin in test samples is determined directly from this calibration curve.

IV. REAGENTS: Preparation and Storage

The Eagle Biosciences Humanized Anti-HER-2 (Herceptin/Trastuzumab) ELISA Assay kit must be stored at 2 – 8 °C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components are stable until this expiration date.

Prior to use allow all reagents to come to room temperature. Regents from different kit lot numbers should not be combined or interchanged.

1. **HER-2 Coated Microplate**
   One microplate with twelve by eight strips (96 wells total) coated with recombinant HER-2 protein. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

2. **Herceptin Tracer Antibody**
   One vial containing **12 mL** concentrated Tracer Antibody in a stabilized protein matrix. This reagent should be stored at 2 – 8 °C and is stable until expiration date on the kit box.

3. **ELISA Wash Concentrate**
   One bottle contains **30 mL** of 30-fold concentrate. Before use the contents must be diluted with **870 mL** of demineralized water and mixed well. Upon dilution, this yields a working wash solution containing a surfactant in phosphate-buffered saline with a non-azide, non-mercury preservative. The diluted wash solution may be stored at room temperature and is stable until the expiration date on the kit box.

4. **ELISA HRP Substrate**
   One bottle contains **12 mL** of ready-to-use tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

5. **ELISA Stop Solution**
   One bottle contains **15 mL** of 1N Sulfuric Acid. This reagent may be stored at 2 – 8 °C or room temperature and is stable until the expiration date on the kit box. **Caution: this component contains potentially hazardous material.**

6. **Herceptin Calibrators**
   Six vials containing ready-to-use Herceptin in a serum based matrix with a non-azide preservative. **Refer to vials for exact concentration for each standard.** These reagents should be stored at 2-8 °C, -20°C for longer storage, prior to reconstitution and are stable until the expiration date on the kit box.

7. **Herceptin Controls**
   Two vials containing ready-to-use Herceptin in serum based matrix with non-azide preservative. **Refer to vials for exact concentration range for each control.** Both controls should be stored at 2-8 °C, -20°C for longer storage, prior to reconstitution and are stable until the expiration date on the kit box.

8. **Herceptin Concentrated Assay Buffer**
   One bottle containing **30 mL** of **4-fold** concentrated buffer matrix with protein stabilizers and preservative. This reagent should be stored at 2-8°C and is stable until the expiration date on the kit box. Before use the concentrated buffer must be diluted with **90 mL** of demineralized water and mixed well. Upon dilution, this

yields as **1x Assay Buffer**, which serves as the **patient sample diluent** containing a surfactant in phosphate-buffered saline with a non-azide preservative. The diluted reagent is stored at 2-8°C and is stable until expiration date on the kit box.

V. SAFETY PRECAUTIONS

The reagents must be used in a professional setting by trained personnel. The source material for reagents containing bovine serum was derived in the contiguous 48 United States. It was obtained only from healthy donor animals maintained under veterinary supervision and found free of contagious diseases.

Wear gloves while performing this assay and handle these reagents as if they are potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or hydrochloric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Hydrochloric acid may cause severe irritation on contact with skin. Provide good ventilation in process area to prevent formation of vapor. Do not breathe mist, vapors, spray. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices.

VI. MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 50 µL, 100 µL, 1,000 µL, etc.
2. Disposable pipette tips suitable for above volume dispensing.
3. Disposable plastic 100 mL and 1000 mL bottle with caps.
4. Aluminum foil.
5. Deionized or distilled water.
6. Plastic microtiter well cover or polyethylene film.
7. ELISA multi-channel wash bottle or automatic (semi-automatic) washing system.
8. Spectrophotometric microplate reader capable of reading absorbance at 450 nm and 650 or 630

VII. SPECIMEN COLLECTION

Serum or EDTA plasma are acceptable samples.
Only **10 µL** of sample is required for a duplicate determination of active Herceptin with this test kit. No special preparation of individual is necessary prior to specimen collection. Samples should be collected by calibrator methods of clinical laboratory practice and recommended by the manufacturer of the sample collection tube. It is extremely important to carefully separate the plasma from blood cells to avoid hemolyzation, etc. Samples should be transferred to a clean test tube right after centrifugation and should be stored at 2 – 8 ºC if the assay is to be performed within 72 hours. Otherwise, patient samples should be stored at - 20 ºC or below until measurement. Avoid more than three times freeze-thaw cycles of specimen. Do not use hemolyzed, hyperlipermic, heat-treated or any contaminated specimens.

VIII. ASSAY PREPARATION

1. Reagent Preparation

1. Prior to use allow all reagents to come to room temperature. Reagents from different kit lot numbers should not be combined or interchanged.
2. ELISA Wash Concentrate, and Herceptin Concentrated Assay Buffer must be diluted to working solution prior to use. Please see REAGENTS section for details.
3. **Each unknown sample needs to be diluted 1:100 using the 1x Assay Buffer.**
4. Place a sufficient number of HER-2 coated microwell strips in a holder to determine calibrators and diluted unknown samples in duplicates.

VIII. ASSAY PROCEDURE

Test Configuration

Row	STRIP 1	STRIP 2	STRIP 3
A	CAL 1	CAL 5	SAMPLE 1
B	CAL 1	CAL 5	SAMPLE 1
C	CAL 2	CAL 6	SAMPLE 2
D	CAL 2	CAL 6	SAMPLE 2
E	CAL 3	CTL 1	SAMPLE 3
F	CAL 3	CTL 1	SAMPLE 3
G	CAL 4	CTL 2	SAMPLE 4
H	CAL 4	CTL 2	SAMPLE 4

1. Add **25 µL** of calibrators, controls and **diluted 1:100** test samples into the designated microwells.
2. Immediately add **100 µL** of working 1x assay buffer into the designated microwells.
3. Seal the plate wells securely, cover with foil or other material to protect from light and rotate on an ELISA plate shaker (small orbit radius) for **1 hour** at 400 to 450 rpm.
4. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
5. Add 100 µL of Herceptin Tracer Antibody to each well.
6. Seal the plate wells securely, cover with foil or other material to protect from light and rotate for **30 minutes** on an ELISA plate shaker (small orbit radius) at 400 to 450 rpm.
7. Wash each well 5 times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
8. Add **100 µL** of ELISA HRP Substrate into each of the wells.
9. Cover the plate with aluminum foil or other material to avoid exposure to light. Incubate plastic static, at room temperature for **20 minutes**.

10. Immediately add **100 µL** of ELISA Stop Solution into each of the wells. Mix gently.
11. Read the absorbency at 450 nm.

X. PROCEDURAL NOTES

1. It is recommended that all calibrators, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light-sensitive reagents in the original amber bottles.
3. Store any unused antibody-coated strips in the foil zipper bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. An orbital mixer with a larger orbit radius (e.g. >1 cm) may be used at speeds of 150 to 200 rpm.
7. Avoid introducing air bubbles into the microwells as this could result in lower binding efficiency and higher CV% of duplicate reading.
8. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.
9. If adapting this assay to automated ELISA system such as DS-2 or EuChrom DUO a procedural validation is necessary if there is any modification of the assay procedure.

XI. INTERPRETATION OF RESULTS

It is recommended to use a point-to-point calibration curve fitting.

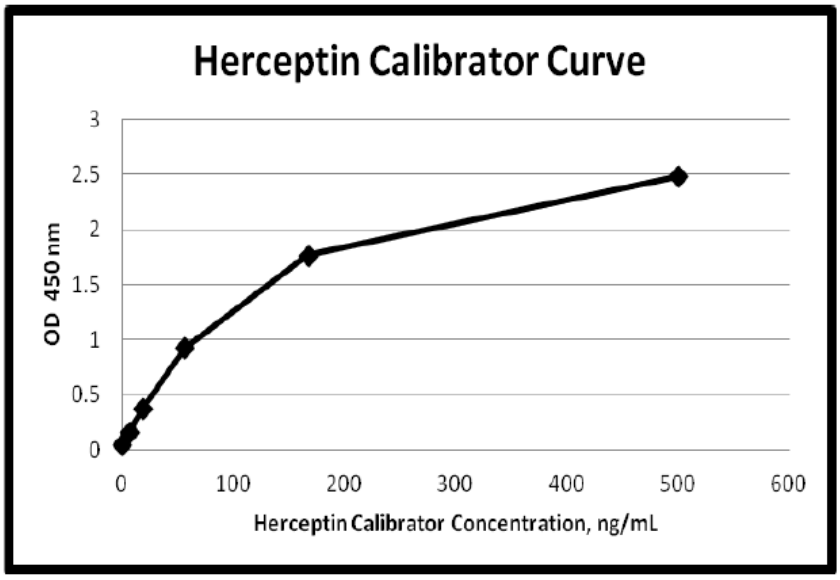
1. Calculate the average absorbance for each pair of duplicate test results.
2. The calibration curve is generated by the corrected absorbance of all calibrator levels on the ordinate against the calibrator concentration on the abscissa using a cubic plot. Appropriate computer assisted data reduction programs may also be used for the calculation of results.

The Herceptin calibrator and control concentrations for the test samples are read directly from the calibrator curve using their respective corrected absorbance.

XII. EXAMPLE DATA AND STANDARD CURVE

A typical absorbance data and the resulting standard curve from this AMH ELISA are represented. **This curve should not be used in lieu of standard curve generated with each assay.**

Well I.D.	OD 450/650 nm Absorbance			Results
	Readings	Average	Corrected	
Cal-1 0 ng/mL	0.047 0.047	0.047	0.000	
Cal-2 6.1 ng/mL	0.164 0.166	0.164	0.118	
Cal-3 18.5 ng/mL	0.403 0.360	0.381	0.334	
Cal-4 56 ng/mL	0.946 0.915	0.930	0.883	
Cal-5 167 ng/mL	1.856 1.686	1.771	1.724	
Cal-6 500 ng/mL	2.432 2.534	2.483	2.436	
Control 1	0.300 0.275	0.287	0.240	13.13 ng/ml
Control 2	1.473 1.418	1.445	1.398	124.01 ng/ml



XIII. LIMITATION OF THE PROCEDURE

1. This assay requires human serum or plasma samples for testing.
2. Serum or plasma samples from different species may show different matrix background. A modification of test procedure may be necessary for measuring samples from other species. Please contact Eagle Biosciences for technical support.
3. Cell culture or tissue culture samples should be validated with total binding and other performance specifications before being used.

XIV. QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls.

XV. PERFORMANCE CHARACTERISTICS

Sensitivity
The sensitivity (lowest limit of detection) of this Herceptin ELISA as determined by the corresponding absorbance value of 2-fold calibrator deviation above the mean on 16 determinations of zero calibrator is 0.245 ng/mL.

Specificity
This assay is specific for Herceptin. There are no known interfering substances.

High Dose "hook" effect
This assay has not shown any high does "hook" effect for Herceptin levels up to 1,000 µg/mL.

Precision
The intra-assay precision was validated by measuring three spiked samples with 16 replicate determinations.

Sample #	Mean Value (ng/mL)	CV (%)
1	3.3	6.8
2	25.9	8.0
3	192.6	9.4

The inter-assay precision was validated by measuring two control levels in duplicate in 16 individual assays.

Sample #	Mean Value (mg/mL)	CV (%)
1	12.9	7.3
2	119.9	7.3

Linearity
Three samples were diluted with calibrator zero and tested. The results of Herceptin dilution recovery value are as follows:

Dilution	Observed Value (ng/mL)	Recovery %
Sample A (1:100)	100.0	-
1:2	46.7	93.4
1:4	22.7	90.8
1:8	10.6	84.3
Sample B (1:100)	99.0	-
1:2	46.8	94.6
1:4	23.3	94.3
1:8	10.4	84.3
Sample C (1:100)	0.500	-
1:2	0.250	100.0
1:4	0.122	97.6
1:8	0.066	105.6

Spike Recovery
Three samples are equal volume mixed with calibrators level 3,4,5 and tested. The results are as follows:

Spiked Sample	Observed Value (ng/mL)	Recovery %
Sample A (1:100)	0.2	-
Cal 3: 18.5 ng/mL	9.2	98.3
Cal 4: 56 ng/mL	31.1	110.5
Cal 5: 167 ng/mL	89.9	107.5
Sample B (1:100)	0.5	-
Cal 3: 18.5 ng/mL	12.7	133.7
Cal 4: 56 ng/mL	29.4	104.2
Cal 5: 167 ng/mL	98.9	118.0
Sample C (1:100)	0.6	-
Cal 3: 18.5 ng/mL	8.3	87.2
Cal 4: 56 ng/mL	27.0	95.2
Cal 5: 167 ng/mL	80.3	95.8

XVI. REFERENCES

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3. Journal of Clinical Oncology (Impact Factor: 18.04). 11/2009; 28(1):92-8. DOI:10.1200/JCO.2008.19.9844, The University of Texas M. D. Anderson Cancer Center.
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Herceptin ELISA: Condensed Assay Protocol

1. **25 µl calibrators and Diluted (1:100) unknown samples**

   + *Immediately*

   100µl 1x Assay Buffer

   ↓ *Incubate @ RT for 1 hour on ELISA plate shaker Wash 5x*

2. **100 µl Tracer Antibody**

   ↓ *Incubate @ RT for 30 min on ELISA plate shaker Wash 5 x*

3. **100 µl TMB Substrate**

   ↓ *Incubate @ RT for 20 min static*

4. **100 µl Stop Solution** *Immediately*

   ↓

5. **Read absorbance at 450 nm** *within 10 minutes*

Warranty Information

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.