Epitope Diagnostics, Inc.

EDI™ Herceptin ELISA Kit

Enzyme Linked ImmunoSorbent Assay (ELISA) for the Quantitative Measurement of Herceptin Levels in Serum

REF **KTR-895** Σ 96 +2 +8 °C

For Research Use Only **Not for Use in Diagnostic Procedures**

INTENDED USE
This test kit is intended for use in the quantitative determination of Herceptin levels in human serum or EDTA plasma.

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.

ASSAY PRINCIPLE
The assay utilizes the "sandwich" technique with HER2 coated to solid phase micro titer plate wells and an antibody to human IgG which is labeled with horseradish peroxidase used for detection. Assay calibrators, controls and 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.

REAGENTS: Preparation and Storage
This test 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. **Human HER-2 Coated Microplate (Cat. No. 30755)**
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 (Cat. No. 30756)**
One vial containing **0.6 mL** concentrated Tracer Antibody in a stabilized protein matrix. This reagent should be diluted before use (see reagent preparation for details) and stored at 2 – 8 °C and is stable until the expiration date on the kit box.

3. **Tracer Antibody Diluent (Cat. No. 30710)**
One bottle containing **12 mL** ready-to-use buffer. It should be used only for tracer antibody dilution according to the assay procedure. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

4. **ELISA Wash Concentrate (Cat. No. 10010)**
One bottle containing **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.

5. **ELISA HRP Substrate (Cat. No. 10020)**
One bottle containing **15 mL** of 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.

6. **ELISA Stop Solution (Cat. No. 10030)**
One bottle containing **15 mL** of 1N sulfuric acid. This reagent may be stored at 2 – 8 °C or at room temperature and is stable until the expiration date on the kit box.

7. **Herceptin Calibrators (Cat. No. 30801-30806)**
Six vials containing Herceptin lyophilized in a serum based matrix with a non-azide preservative. **Refer to the vial for exact concentration of the calibrator.** These calibrators should be stored at 2 – 8 °C and are stable until the expiration date on the kit box.

8. **Herceptin Controls (Cat. No. 30807-30808)**
Two vials containing Herceptin lyophilized in a serum based matrix with non-azide preservative. **Refer to the vial for the acceptable concentration ranges for the controls.** These controls should be stored at 2 – 8 °C and are stable until the expiration date on the kit box.

9. **Assay Buffer (Cat. No. 30779)**
One bottle containing **30 mL** of ready-to-use buffer. It should be used according to the assay procedure. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the kit box.

SAFETY PRECAUTIONS

The reagents must be used in professional laboratory. 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 sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. 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.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Precision single channel pipettes capable of delivering 10 µL, 25 µL, 50 µL, 100 µL, etc.
2. Disposable pipette tips suitable for above volume dispensing.
3. Aluminum foil.
4. Deionized or distilled water.
5. Plastic microtiter well cover or polyethylene film.
6. ELISA multi-channel wash bottle or automatic (semi-automatic) washing system.
7. Spectrophotometric microplate reader capable of reading absorbance at 450 nm.

SPECIMEN COLLECTION

Serum or EDTA-plasma samples are suitable specimens for active Herceptin measurement. 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 standard 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.

ASSAY PROCEDURE

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 (Cat. 10010) must be diluted to working solution prior to use. Please see REAGENTS section for details.

(3) Reconstitute all assay standards (Cat. 30801-30806) and controls (Cat. 30807-30808) by adding **0.5 mL** of deminerialized water to each vial. Allow the standards and controls to sit undisturbed for 10 minutes, and then mix well by inversions or gentle vortexing. Make sure that all solids are dissolved completely prior to use. These reconstituted standards and controls must be stored at -10 °C or below. Do not exceed 3 freeze-thaw cycles.

(4) Each unknown sample needs to be diluted 1:100 using the Assay Buffer.

(5) Prepare Herceptin Tracer Antibody working solution by **1:21** fold dilution of the Herceptin Tracer Antibody (Cat. 30756) by adding the Tracer Antibody into the Tracer Antibody Diluent (Cat. 30710). Following is a table that outlines the relationship of strips used and antibody mixture prepared. ***NOTE:*** *the Tracer Antibody should be prepared just prior to use.*

Dilution Scheme	Tracer Antibody Diluent	Tracer Antibody
1	1 mL	50 µL
2	2 mL	100 µL
3	3 mL	150 µL
4	4 mL	200 µL
5	5 mL	250 µL
6	6 mL	300 µL
7	7 mL	350 µL
8	8 mL	400 µL
9	9 mL	450 µL
10	10 mL	500 µL
11	11 mL	550 µL
12	12 mL	600 µL

(6) Place a sufficient number of HER-2 coated microwell strips in a holder to determine calibrators and diluted unknown samples in duplicates.

2. Assay Procedure:

(1) Test Configuration

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

(1) Add **25 µL** of calibrators, controls, and **diluted** test samples into the designated microwells. Tap the plate gently.

(2) Immediately add 100 µL of 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 diluted Tracer Antibody (cat# 30756) to each well. Tap the plate gently.

(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 1**00 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 plate 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 absorbance at 450 nm.

PROCEDURAL NOTES

1. It is recommended that all standards 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. It is recommended to add external controls to each assay.
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 air bubbles in the microwell 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, DSX or Trituras, a procedural validation is necessary if there is any modification of the assay procedure.

INTERPRETATION OF RESULTS

It is recommended to use a point to point standard curve fitting.

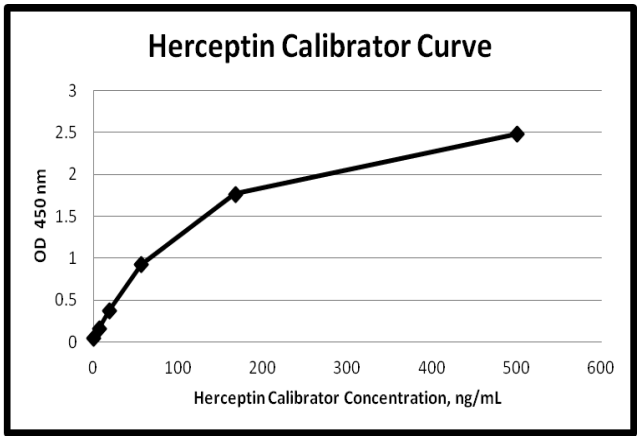
1. Calculate the average absorbance for each pair of duplicate test results.
2. The standard curve is generated by the corrected absorbance of all standard levels on the ordinate against the standard concentration. Appropriate computer assisted data reduction programs should be used for the calculation of results.

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

EXAMPLE DATA AND STANDARD CURVE

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

Well I.D.	OD 450 nm Absorbance			Results ng/mL
	Readings	Average	Corrected	
0 ng/mL	0.047 0.047	0.047	0.000	
6.1 ng/mL	0.164 0.166	0.165	0.118	
18.5 ng/mL	0.403 0.360	0.381	0.334	
56 ng/mL	0.946 0.915	0.930	0.883	
167 ng/mL	1.856 1.686	1.771	1.724	
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



LIMITATION OF THE PROCEDURE

1. This assay requires human serum or plasma sample 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 Epitope Diagnostics for technical support.
3. Cell culture or tissue culture samples should be validated with total binding and other performance specifications before being used.

QUALITY CONTROL

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

PERFORMANCE CHARACTERISTICS

Sensitivity
The sensitivity (lowest limit of detection) of this Herceptin ELISA as determined by the corresponding absorbance value of 2-fold standard 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 dose "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 (ng/mL)	CV (%)
1	12.9	7.3
2	119.9	7.3

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

DILUTION	OBSERVED VALUE (ng/mL)	RECOVERY %
Neat A	100.1	-
1:2	46.7	93.4
1:4	22.7	90.8
1:8	10.6	84.3
Neat B	99.0	-
1:2	46.8	94.6
1:4	23.3	94.3
1:8	10.4	84.3
Neat C	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 standard level 3, 4, 5 and tested. The results are as follows:

Spiked Sample	OBSERVED VALUE (ng/mL)	RECOVERY %
Neat A	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
Neat B	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
Neat C	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

WARRANTY

This product is warranted to perform as described in its labeling and literature when used in accordance with all instructions. Epitope Diagnostics, Inc. DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE, and in no event shall Epitope Diagnostics, Inc. be liable for consequential damages. Replacement of the product or refund of the purchase price is the exclusive remedy for the purchaser. This warranty gives you specific legal rights and you may have other rights, which vary from state to state.

REFERENCES

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TECHNICAL ASSISTANCE AND CUSTOMER SERVICE
For technical assistance or place an order, please contact Epitope Diagnostics, Inc. at (858) 693-7877 or fax to (858) 693-7678.
www.epitopediagnostics.com

This product is developed and manufactured by
Epitope Diagnostics, Inc.
San Diego, CA 92121, USA

Manufacturer	No. of tests
Catalog Number	Keep away from heat and direct sun light
Concentrate	Store at
Read instructions before use	Use by
	Lot No.

Herceptin ELISA: Condensed Assay Protocol

1. 25 µl standards and
Diluted unknown samples

+ *Immediately*

100µl 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*