Quantikine® ELISA

Human ErbB2/Her2 Immunoassay

Catalog Number DHER20

For the quantitative determination of human ErbB2 concentrations in cell culture supernates, cell lysates, serum, plasma, urine, and human milk.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

ErbB2, also called Neu and Her2 (Human Epidermal Growth Factor Receptor 2), is a type I membrane glycoprotein member of the ErbB family of tyrosine kinase receptors (1, 2). Human ErbB2 is 1255 amino acids in length with a molecular mass of 185 kDa (3). ErbB family members serve as receptors for the epidermal growth factor (EGF) family of growth factors. ErbB2 is widely expressed in epithelial cells. It is unique in that it has no identified ligands, rather, ErbB2 heterodimerizes with other members of the ErbB family (ErbB1/EGF R, ErbB3, ErbB4) to form ligand-activated signaling complexes (4). ErbB2 is involved in cardiovascular homeostasis, communication at the neuromuscular junction, and in the development and progression of cancer (5-10).

Soluble forms of ErbB2 can be generated by proteolytic shedding or by alternative splicing (11, 12). Herstatin is a secreted alternatively-spliced ErbB2 gene product that contains a portion of the ErbB2 extracellular domain as well as an intron-encoded sequence. Herstatin can bind to ErbB1 and ErbB2 to block receptor heterodimerization and signaling (11, 13, 14). In addition to Herstatin secretion, the extracellular domain (ECD) of full-length ErbB2 can be cleaved by ADAM10 and shed as a soluble protein (15). Cleavage of the ErbB2 ECD results in a kinase-active truncated protein, $p95^{Her2}$, which is composed of the transmembrane and cytoplasmic domains and has enhanced oncogenic potency (16, 17).

Serum levels of cleaved soluble ErbB2 are elevated in individuals with $Her2^{+}$ metastatic breast tumors and may be associated with survival (12, 18). Treatment with Trastuzumab, a humanized anti-ErbB2 monoclonal antibody, may inhibit ErbB2 shedding resulting in decreased serum ErbB2 levels (19). Alternatively, treatment with Lapatinib, a small molecule tyrosine kinase inhibitor, may increase ErbB2 shedding (20).

The Quantikine Human ErbB2/Her2 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human ErbB2 in cell culture supernates, cell lysates, serum, plasma, urine, and human milk. It contains NS0-expressed recombinant human ErbB2 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human ErbB2 showed linear curves that were parallel to the standard curves obtained using the Quantikine kit standards. These results indicate that this kit can be used to determine relative mass values for naturally occurring human ErbB2.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human ErbB2 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ErbB2 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human ErbB2 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of ErbB2 bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, further dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human ErbB2 Microplate	894678	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human ErbB2.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human ErbB2 Conjugate	894679	21 mL of a polyclonal antibody specific for human ErbB2 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human ErbB2 Standard	894680	Recombinant human ErbB2 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:4 in this assay.*	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human ErbB2 Controls (optional; R&D Systems, Catalog # QC86).

SUPPLIES REQUIRED FOR CELL LYSATE SAMPLES

- Cell Lysis Buffer 2 (R&D Systems, Catalog # 895347)
- PBS

PRECAUTIONS

ErbB2 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Please refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Cell Lysates - Cells must be lysed prior to assay as directed in the Cell Lysis Procedure.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature 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 or heparin 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.

Note: *Citrate plasma has not been validated for use in this assay.*
Grossly hemolyzed samples are not suitable for use in this assay.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Human Milk - Centrifuge for 15 minutes at 1000 x g at 2-8 °C. Collect the aqueous fraction and repeat this process a total of 3 times. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples require a 10-fold dilution. A suggested 10-fold dilution is 20 µL of sample + 180 µL of Calibrator Diluent RD5-26 (diluted 1:4).*

CELL LYSIS PROCEDURE

Use the following procedure for the preparation of cell lysate samples.

1. Wash cells three times in cold PBS.
2. Resuspend cells at 1 x 10^7 cells/mL in Cell Lysis Buffer 2.
3. Incubate with gentle agitation for up to 60 minutes at room temperature.
4. Centrifuge at 8000 x g for 10 minutes to remove cell debris.
5. Assay immediately or aliquot the lysis supernates and store at ≤ -70 °C until ready for use.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *ErbB2 is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

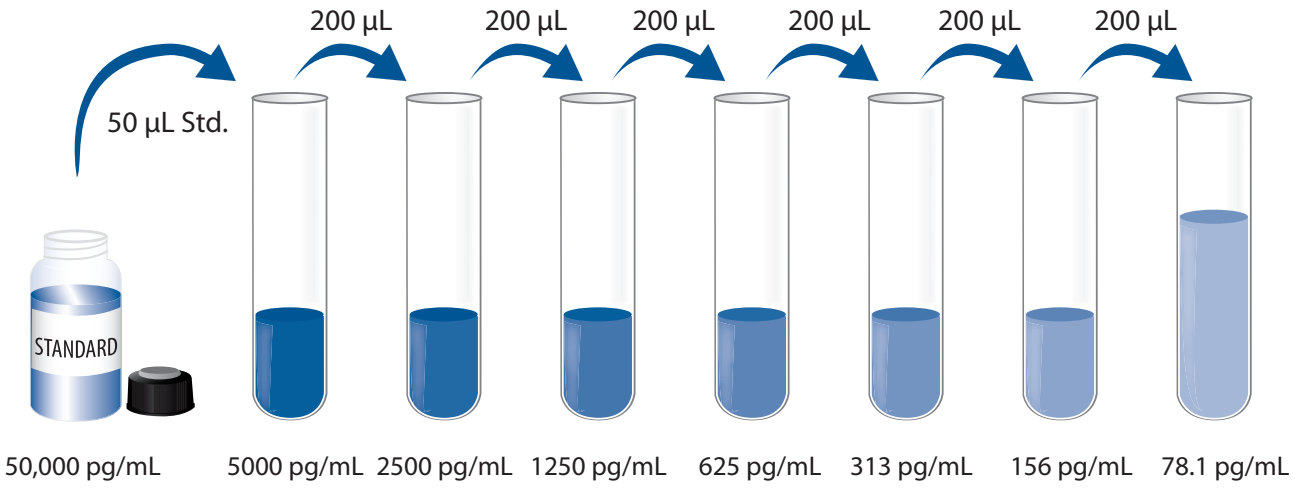
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5-26 (diluted 1:4) - Add 20 mL of Calibrator Diluent RD5-26 Concentrate to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (diluted 1:4).

Human ErbB2 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human ErbB2 Standard with deionized or distilled water. This reconstitution produces a stock solution of 50,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD5-26 (diluted 1:4) into the 5000 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 5000 pg/mL standard serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all samples, controls, and standards be assayed in duplicate.

Note: *ErbB2 is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1W to each well.
4. Add 50 µL of Standard, control, or sample* per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human ErbB2 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

*Samples may require dilution. See the Sample Preparation section.

CALCULATION OF RESULTS

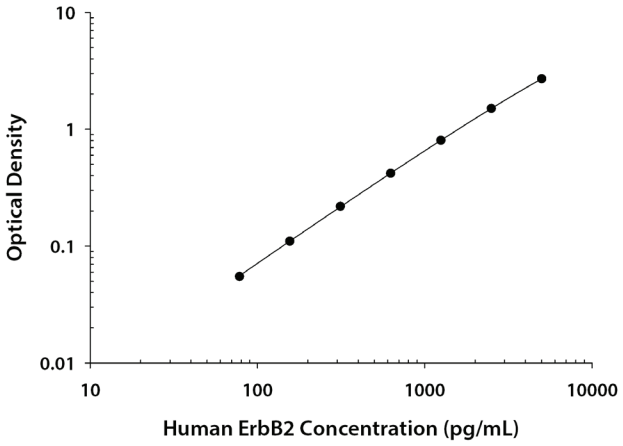
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human ErbB2 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

TYPICAL DATA

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



(pg/mL)	O.D.	Average	Corrected
0	0.023 0.027	0.025	—
78.1	0.079 0.081	0.080	0.055
156	0.134 0.135	0.135	0.110
313	0.244 0.244	0.244	0.219
625	0.442 0.445	0.444	0.419
1250	0.824 0.834	0.829	0.804
2500	1.528 1.532	1.530	1.505
5000	2.720 2.727	2.724	2.699

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	471	1379	2681	489	1419	2667
Standard deviation	6.71	16.3	31.1	18.7	56.4	93.5
CV (%)	1.4	1.2	1.2	3.8	4.0	3.5

RECOVERY

The recovery of human ErbB2 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	103	90-120%
Serum* (n=4)	99	91-115%
EDTA plasma* (n=4)	99	89-118%
Heparin plasma* (n=4)	104	92-119%
Urine (n=4)	101	94-106%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Thirty-one assays were evaluated and the minimum detectable dose (MDD) of human ErbB2 ranged from 2.33-14.8 pg/mL. The mean MDD was 4.86 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human ErbB2 were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Cell lysates* (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Urine (n=4)	Human milk (n=4)
1:2	Average % of Expected	98	102	102	102	106	98	100
	Range (%)	96-100	99-107	99-104	101-106	104-108	97-99	96-105
1:4	Average % of Expected	96	102	104	104	109	96	105
	Range (%)	95-97	95-110	99-106	102-108	106-111	94-98	102-108
1:8	Average % of Expected	97	102	105	105	110	95	105
	Range (%)	96-100	97-109	100-108	103-110	107-112	91-99	102-108
1:16	Average % of Expected	95	100	104	104	109	92	103
	Range (%)	94-97	95-104	97-108	102-110	103-112	89-96	101-104

*Samples were diluted prior to assay.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human ErbB2/Her2 manufactured at R&D Systems.

SAMPLE VALUES

Serum/Plasma/Urine/Human Milk - Samples from apparently healthy volunteers were evaluated for the presence of human ErbB2 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=36)	5520	3884-7754	911
EDTA plasma (n=36)	5296	3757-7475	974
Heparin plasma (n=36)	5248	3756-7461	908
Human milk (n=10)	1155	727-2152	459

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Urine (n=11)	103	36	ND-123

ND=Non-detectable

Cell Culture Supernates/Cell Lysates:
MCF-7 human breast cancer cells were cultured in DMEM/Kaugn's supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate, and grown until confluent.

MDA-MB-453 human breast cancer cells were cultured in RPMI supplemented with 10% fetal bovine serum and 2 mM L-glutamine, and grown until confluent.

SK-OV-3 human ovarian adenocarcinoma cells were cultured in McCoy's 5A supplemented with 10% fetal bovine serum and grown until confluent.

OVCAR-3 human ovarian carcinoma cells were cultured in RPMI supplemented with 10% fetal bovine serum and 0.25 µg/mL insulin, and grown until confluent.

An aliquot of each cell culture supernate was removed and assayed for human ErbB2. The cells were lysed according to the Cell Lysis Procedure and assayed for human ErbB2. Cell lysate was normalized to total protein concentration.

Cell Line	Cell Culture Supernate Values (pg/mL)	Cell Lysate Values (pg/mg)
MCF-7	261	3893
MDA-MB-453	2259	66,471
SK-OV-3	343	572,066
OVCAR-3	162	2269

SPECIFICITY

This assay recognizes natural and recombinant human ErbB2.

The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human ErbB2 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

EGF R/ErbB1
ErbB3
ErbB4
FAK
GRB2
L1CAM
SOS2

Recombinant mouse:

ErbB2/Her2

Recombinant human ErbB2 Herstatin Isoform cross-reacts approximately 8% in this assay.

REFERENCES

1. Coussens, L. *et al.* (1985) Science **230**:1132.
2. Schechter, A.L. *et al.* (1985) Science **229**:976.
3. Akiyama, T. *et al.* (1986) Science **232**:1644.
4. Penuel, E. *et al.* (2001) Semin. Oncol. **28**:36.
5. Iwamoto, R. and E. Mekada (2006) Cell Struct. Funct. **31**:1.
6. Ponomareva, O.N. *et al.* (2006) Mol. Cell. Neurosci. **31**:334.
7. Waage, I.S. *et al.* (2013) Int. J. Surg. Pathol. **21**:573.
8. Jelovac, D. and L.A. Emens (2013) Oncology (Williston Park) **27**:166.
9. Korkaya, H. and M.S. Wicha (2013) Cancer Res. **73**:3489.
10. Rexer, B.N. and C.L. Arteaga (2013) Cancer Res. **73**:3817.
11. Justman, Q.A. and G.M. Clinton (2002) J. Biol. Chem. **277**:20618.
12. Tse, C. *et al.* (2012) Cancer Treat. Rev. **38**:133.
13. Azios, N.G. *et al.* (2001) Oncogene **20**:5199.
14. Doherty, J.K. *et al.* (1999) Proc. Natl. Acad. Sci. USA **96**:10869.
15. Liu, P.C. *et al.* (2006) Cancer Biol. Ther. **5**:657.
16. Christianson, T.A. *et al.* (1998) Cancer Res. **58**:5123.
17. Segatto, O. *et al.* (1988) Mol. Cell. Biol. **8**:5570.
18. Moreno-Aspitia, A. *et al.* (2013) Cancer **119**:2675.
19. Molina, M.A. *et al.* (2001) Cancer Res. **61**:4744.
20. Vazquez-Martin, A. *et al.* (2011) J. Cell. Physiol. **226**:52.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.