

# Quantikine<sup>®</sup> ELISA

## Human ErbB3/Her3 Immunoassay

Catalog Number DERB30

For the quantitative determination of human ErbB3 concentrations in cell culture supernates, cell lysates, serum, plasma, saliva, 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

ErbB3, also called Her3 (Human Epidermal Growth Factor Receptor 3), is a type I membrane glycoprotein that is a member of the ErbB family of tyrosine kinase receptors (1). ErbB family members serve as receptors for the epidermal growth factor (EGF) family of ligands. Among the ErbB tyrosine kinase receptors, ErbB3 is unique in that, although it can bind its ligand, a defective kinase domain weakens its intrinsic kinase activity and signaling into the cell. Rather, ErbB3 forms heterodimers with other ErbB family members to elicit ligand-activated signaling cascades (2). The pathways activated promote cell proliferation and differentiation; as such, overexpression of ErbB3 has been implicated in various cancer types (3-5).

Human ErbB3 consists of 1342 amino acids (aa) with a 19 aa signal sequence, a 624 aa extracellular domain, a 21 aa transmembrane region, and a 678 aa cytoplasmic domain. Human ErbB3 has four isoforms created by intron read-through and truncation, three of which are secreted and at least one can modulate membrane-bound ErbB3 activity (6-8). Secreted ErbB3 as a serum biomarker for human disease has been reported, however, not thoroughly characterized (9,10).

The Quantikine® Human ErbB3/Her3 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human ErbB3 in cell culture supernates, cell lysates, serum, plasma, saliva, urine and human milk. It contains NS0-expressed recombinant human ErbB3 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human ErbB3 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 natural human ErbB3.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human ErbB3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any ErbB3 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human ErbB3 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 ErbB3 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 the calibrator diluent and repeat the assay.
- Any variation in 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 ErbB3 Microplate	899466	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human ErbB3.	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 ErbB3 Standard	899468	2 vials of recombinant human ErbB3 in a buffered protein solution with preservatives; lyophilized. <i>Refer to the vial label for reconstitution volume.</i>	Use a fresh standard for each assay. Discard after use.
Human ErbB3 Conjugate	899467	21 mL of a polyclonal antibody specific for human ErbB3 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1N	895488	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a concentrated buffered protein base with preservatives. <i>Use diluted 1:4 in this assay.</i>	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. <i>May turn yellow over time.</i>	
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
- 50 mL and 500 mL graduated cylinder
- 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 ErbB3 Controls (optional; R&D Systems®, Catalog # QC275)

### If using cell lysate samples, the following are also required:

- Cell Lysis Buffer 17 (R&D Systems®, Catalog # 895943)
- PBS

## PRECAUTIONS

ErbB3 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. Refer to the SDS 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  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Cell Lysates** - Cells must be lysed prior to assay as directed in the Sample Values section.

**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  $\leq -20$  °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  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

**Note:** *Citrate plasma has not been validated for use in this assay.*

**Saliva** - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, and assay immediately or aliquot and store samples at  $\leq -20$  °C. Avoid repeated freeze-thaw cycles.

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

## SAMPLE PREPARATION

Serum, plasma, saliva, and human milk samples require a 10-fold dilution due to high endogenous levels. A suggested 10-fold dilution is 20  $\mu\text{L}$  of sample + 180  $\mu\text{L}$  of Calibrator Diluent RD5-26 (diluted 1:4).

The suggested range for total cell lysate protein to add is 5-50  $\mu\text{g}/\text{well}$ .

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**

**Note:** *ErbB3* is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.

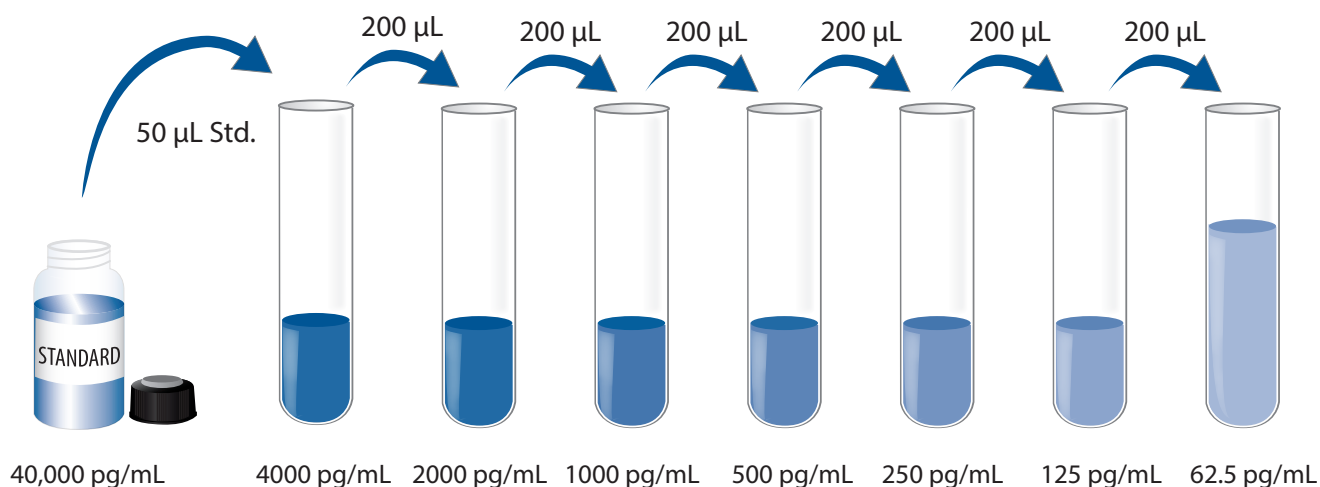
**Wash Buffer** - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

**Substrate Solution** - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200  $\mu\text{L}$  of the resultant mixture is required per well.

**Calibrator Diluent RD5-26 (diluted 1:4)** - Add 10 mL of Calibrator Diluent RD5-26 Concentrate to 30 mL of deionized or distilled water to prepare 40 mL of Calibrator Diluent RD5-26 (diluted 1:4).

**Human ErbB3 Standard - Refer to the vial label for reconstitution volume.** Reconstitute the Human ErbB3 Standard with deionized or distilled water. This reconstitution produces a stock solution of 40,000 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  $\mu\text{L}$  of Calibrator Diluent RD5-26 (diluted 1:4) into the 4000 pg/mL tube. Pipette 200  $\mu\text{L}$  into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4000 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 standards, controls, and samples be assayed in duplicate.**

**Note:** *ErbB3 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  $\mu\text{L}$  of Assay Diluent RD1N to each well.
4. Add 50  $\mu\text{L}$  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  $500 \pm 50$  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  $\mu\text{L}$ ) 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  $\mu\text{L}$  of Human ErbB3 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  $\mu\text{L}$  of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50  $\mu\text{L}$  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 Sample Preparation section.

## CALCULATION OF RESULTS

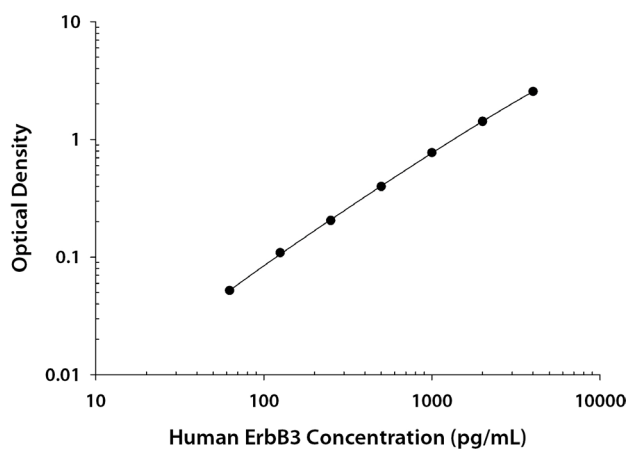
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 ErbB3 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	0.021 0.026	0.024	—
62.5	0.073 0.079	0.076	0.052
125	0.126 0.140	0.133	0.109
250	0.228 0.229	0.229	0.205
500	0.423 0.423	0.423	0.399
1000	0.790 0.803	0.797	0.773
2000	1.444 1.450	1.447	1.423
4000	2.555 2.612	2.584	2.560

## 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.

Sample	Intra-Assay Precision			Inter-Assay Precision		
	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	396	1176	2446	437	1317	2646
Standard deviation	4.56	11.2	36.9	29.4	66.1	119
CV (%)	1.2	1.0	1.5	6.7	5.0	4.5

## RECOVERY

The recovery of human ErbB3 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	97	92-100%
Cell lysis buffer (n=2)	98	96-101%
Serum* (n=4)	100	93-109%
EDTA plasma* (n=4)	97	93-103%
Heparin plasma* (n=4)	96	91-102%
Saliva* (n=4)	100	94-106%
Urine (n=4)	97	94-102%
Human milk* (n=4)	96	91-103%

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

## SENSITIVITY

Twenty-one assays were evaluated and the minimum detectable dose (MDD) of human ErbB3 ranged from 2.11-15.2 pg/mL. The mean MDD was 4.99 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. 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 ErbB3 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Cell lysates (n=4)	Serum* (n=4)	EDTA plasma* (n=4)
1:2	Average % of Expected	102	101	99	99
	Range (%)	99-105	99-103	97-100	97-101
1:4	Average % of Expected	102	101	99	99
	Range (%)	97-104	96-107	97-100	97-101
1:8	Average % of Expected	100	102	98	98
	Range (%)	99-103	100-109	96-100	96-102
1:16	Average % of Expected	90	100	98	98
	Range (%)	86-93	90-108	94-102	95-101

\*Samples were diluted prior to assay.

		Heparin plasma* (n=4)	Saliva* (n=4)	Urine (n=4)	Human milk* (n=4)
1:2	Average % of Expected	100	98	102	101
	Range (%)	100-100	97-100	100-103	100-103
1:4	Average % of Expected	100	98	101	101
	Range (%)	99-101	96-101	100-102	99-104
1:8	Average % of Expected	100	99	101	102
	Range (%)	98-102	97-101	100-102	94-114
1:16	Average % of Expected	97	98	101	95
	Range (%)	88-101	95-102	98-107	92-96

\*Samples were diluted prior to assay.

## CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human ErbB3/Her3 manufactured at R&D Systems®.

## SAMPLE VALUES

**Serum/Plasma/Urine/Saliva/Human Milk** - Samples from apparently healthy volunteers were evaluated for the presence of human ErbB3 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=30)	10,452	7497-14,278	1665
EDTA plasma (n=30)	9912	7067-13,519	1452
Heparin plasma (n=30)	9977	7255-13,624	1453
Saliva (n=10)	8360	2604-22,586	6816
Urine (n=10)	725	74.3-1202	389
Human milk (n=10)	6246	2251-11,565	2901

### Cell Culture Supernates/Cell Lysates:

MCF-7 human breast cancer cells were cultured in DMEM/Kaighn'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, 2 mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin sulfate and grown until confluent.

MDA-MB-231 human breast cancer cells were cultured in DMEM 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-468 human breast cancer cells were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate, and grown until confluent.

For cell culture supernates, cells along with cell culture conditioned media supernate were centrifuged at 500 x g for 5 minutes. Aliquots of the cell culture supernates were removed and assayed for human ErbB3.

For cell lysates, cells were solubilized in Lysis Buffer 17 and allowed to sit on ice for 30 minutes. Tubes were then centrifuged at 14,000 x g for 5 minutes to remove insoluble material, and the remaining whole cell extract was removed. Whole cell extract protein concentration was quantified using a total protein assay. 10 µg of the cell lysate was removed and assayed for human ErbB3.

Cell Line	Cell Culture Supernate Values (pg/mL)	Cell Lysate Values (pg/mL)
MCF-7	1230	691
MDA-MB-453	1267	2024
MDA-MB-231	ND	ND
MDA-MB-468	582	280

ND=Non-detectable

## SPECIFICITY

This assay recognizes natural and recombinant human ErbB3.

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 ErbB3 control were assayed for interference. No significant cross-reactivity or interference was observed.

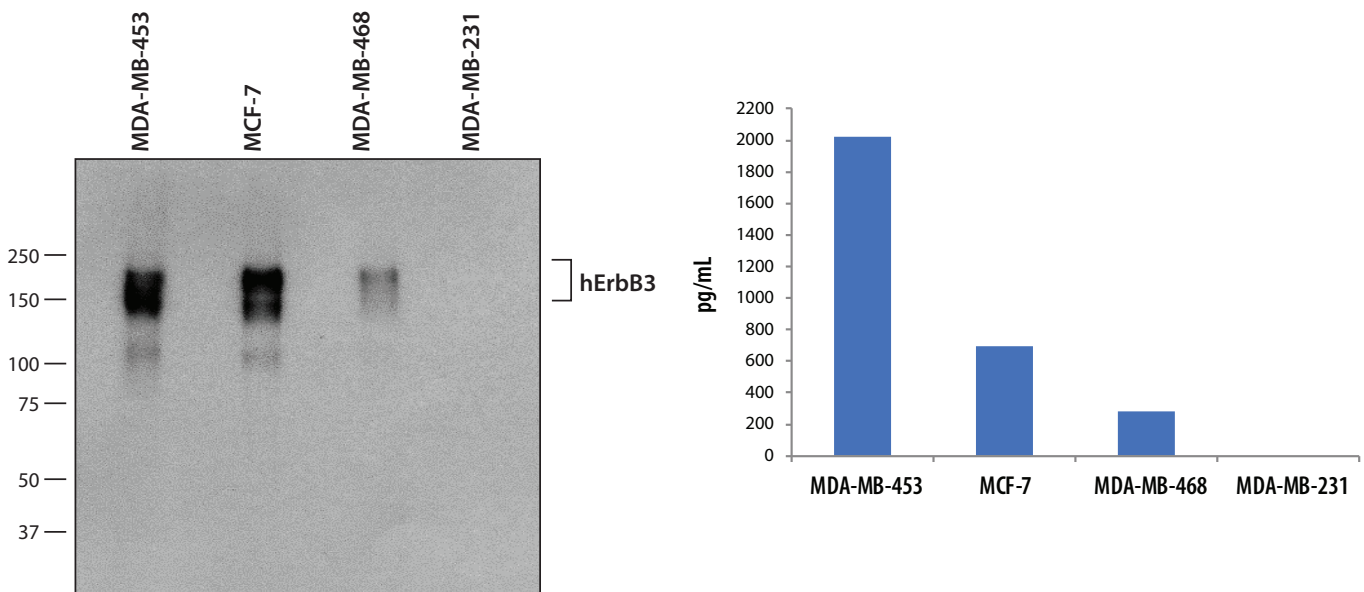
### Recombinant human:

EGFR  
ErbB2  
NRG-1  
NRG-1 $\alpha$   
NRG1- $\beta$ 1  
NRG-2  
NRG-1 Isoform SMDF  
NRG-1 Isoform GGF-2

### Recombinant mouse:

ErbB3

The Human ErbB3/Her3 Quantikine<sup>®</sup> ELISA kit detects human p45-sErbB3 isoform.



Lysates from human MDA-MB-453 cells cultured in RPMI-10%FBS, human MCF-7 cells cultured in DMEM/Kaighn's-10% FBS, and human MDA-MB-468 and MDA-MB-231 cultured in DMEM-10% FBS were analyzed by Western Blot and ELISA. 1.0  $\mu$ g of the cell lysates were resolved for the Western Blot, and 10  $\mu$ g of the cell lysates were tested per well in the ELISA, with the exception of MDA-MB-231 which was tested at 45.5  $\mu$ g per well in the ELISA. Samples were resolved under reducing SDS-PAGE conditions, transferred to PVDF membrane, and immunoblotted with Gt x hErbB3/Her3 (Catalog # AF234). The Western Blot band intensity shows a direct correlation with ELISA sample values.

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# PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

12									
11									
10									
9									
8									
7									
6									
5									
4									
3									
2									
1									
	A	B	C	D	E	F	G	H	

**NOTES**

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