INTENDED USE

The **Human Her2/ErbB2/CD340** ELISA Kit quantifies the Her2 protein, recombinant or native, in cell culture, bioprocessing solutions, and/or in other appropriately qualified samples from tissue fluids (e.g., blood, saliva, mucosa). The assay is not intended for the diagnosis of disease.

GENERAL INFORMATION

HER2 (Human Epidermal Growth Factor Receptor 2), also known as Neu, ErbB-2, CD340 or p185, is amplified or over-expressed in ~30% of breast cancers, and is strongly associated with increased disease recurrence and worse prognosis. Over-expression is also known to occur in ovarian, stomach, and aggressive forms of uterine cancer, such as uterine serous endometrial carcinoma.

The HER2 protein is a transmembrane receptor with an extracellular domain (ECD) and intracellular tyrosine kinase activity. The HER2 ECD is cleaved by a disintegrin and metalloprotease, and the remaining membrane-bound internal domain is constitutively activated. The ECD (p97-115 kDa) of the HER2 protein is released into the circulation; serum HER2 levels are elevated in serum HER2 levels are elevated in 30%-90% of patients with metastatic breast cancer. An elevated pretreatment serum HER2 level is associated with decreased response to both first-line and second-line endocrine therapy. Numerous studies have also shown that changes in serum HER2 parallel the clinical course of disease, with serum HER2 increasing with disease progression and decreasing with treatment response.

HER2 is the target of the monoclonal antibody trastuzumab (Herceptin: by Roche/Genentech). Herceptin, a fully humanized monoclonal antibody (IgG1 kappa), binds to the domain IV of the extracellular segment of the HER2/neu receptor. Herceptin has had a major impact in the treatment of HER2-positive metastatic breast cancer, being mostly effective only in cancers where HER2 is over-expressed.

PRINCIPLE OF THE TEST

The HER2 ELISA kit is based on the binding of HER2 in samples to two antibodies, one immobilized on the microtiter wells, and the other conjugated to horseradish peroxidase (HRP) enzyme. After a washing step, chromogenic substrate is added and color is developed by the enzymatic reaction of HRP on the TMB substrate, which is directly proportional to the amount of HER2 present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microtiter well reader. The concentration of HER2 in samples and control is calculated from a curve of standards containing known concentrations of HER2.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{O}C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **Working Sample Diluent** and store at 2-8ºC until the kit lot expires or is used up.
Wash Solution Concentrate (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Anti-HER2 - HRP Conjugate Concentrate (100x) Part No. 200-533, 0.15ml	Peroxidase conjugated anti-HER2 in buffer with protein, detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8ºC storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
Anti-HER2 Microwell Strip Plate	200-531	8-well strips (12)	Coated with anti-recombinant HER2, and post-coated with stabilizers.
HER2 Standard Part: 200-532	Two (2) vials, each containing HER2 lyophilized in buffer with protein as stabilizers. Keep lyophilized vials refrigerated until used or kit lot expires.		

Freshly reconstitute 1 vial with the volume of **Working Sample Diluent** indicated on the vial to provide a **2** ng/ml solution, sufficient for at least two curves. Prepare 2-fold dilutions, as follows:

Standard	**+**	**Diluent**	**=**	**Final Concn**
Reconstituted Standard		None		**2** ng/ml
225 ul of 2 ng/ml		**225** ul		**1** ng/ml
225 ul of 1 ng/ml		**225** ul		**0.5** ng/ml
225 ul of 0.5 ng/ml		**225** ul		**0.25** ng/ml
225 ul of 0.25 ng/ml		**225** ul		**0.125** ng/ml
225 ul of 0.125 ng/ml		**225** ul		**0.0625** ng/ml

[**Note**: Use within **5 days** of preparation]

Freeze the remaining reconstituted 2ng/ml standard for use within 3 months.

TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Antibody HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate; 0.2 to 1L.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Standards, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid and BND can be requested or obtained from the ADI website: http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

ASSAY DESIGN AND SET-UP

Sample Collection and Handling
Culture medium, bioprocessing preparations, serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference (See Limits of the Assay, page 6). For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature.

For all samples, clarify by centrifugation and/or filtration prior to dilution in Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Assay Validation
Validate the performance of the sample antigen and matrix in the assay system for recovery and parallelism (see Limits of the Assay, page 6), as follows:

Recovery – a measure of the interference of the sample matrix (diluent effect) in providing accurate quantitation of the sample HER2 relative to the Standard curve.

Prepare and run a series of dilutions of the sample antigen (concentrations that will fall within the Standard range) in Working Sample Diluent to determine the dilutions that give consistent and accurate quantitation. For most buffer solutions a minimum 5-fold sample dilution is usually sufficient. Serum and plasma require at least a 20-fold dilution to obtain consistent quantitation or complete antigen recovery.

Parallelism – dilutions of the sample should read equivalent values from the top and bottom of the Standard curve to provide good assay precision.

Prepare a dilution series of the sample antigen that gives complete recovery and falls within the full range of the Standard curve. Sample readings from the upper and lower regions of the curve should differ by less than 25%.

Plate Set-up
Bring all reagents to room temperature (18-30^{o} C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 12 Standard wells and 2 wells for each sample and control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of calibrators, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 60 min; 5 washes]

- Add 100ul of diluted Anti-HER2 HRP Conjugate to each well.
- Incubate for 60 minutes.
- Wash wells 5 times as in step 2.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

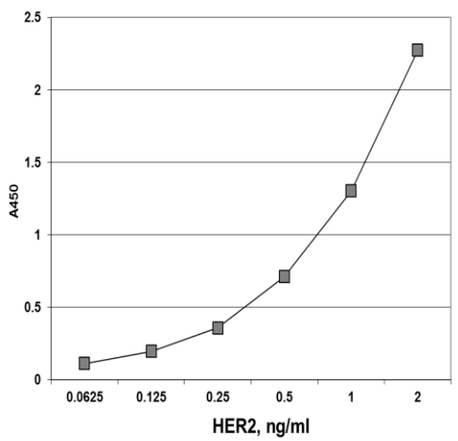
CALCULATION OF RESULTS

1. The results may be calculated using any immunoassay software package. The four-parameter curve-fit is recommended. If software is not available, HER2 concentrations may be determined as follows:
2. Calculate the mean OD of duplicate samples.
3. On graph paper plot the mean OD of the standards (y-axis) against the concentration (ng/ml) of HER2 (x-axis). Draw the best fit curve through these points to construct the standard curve. A point-to-point construction is most common and reliable.
4. The HER2 concentrations in unknown samples and controls can be determined by interpolation from the standard curve.
5. Multiply the values obtained for the samples by the dilution factor of each sample.
6. Samples producing signals higher than the 2 ng/ml standard should be further diluted and re-assayed.

Typical Results:

Wells	Standard Dilutions Samples		**A450 nm**
A1, A2	Diluent Blank		0.10
B1, B2	0.0625 ng/ml	**Standard**	0.23
C1, C2	0.125 ng/ml	**Standard**	0.55
D1, D2	0.25 ng/ml	**Standard**	0.95
E1, E2	0.5 ng/ml	**Standard**	1.84
F1, F2	1.0 ng/ml	**Standard**	2.60
F1, F2	2.0 ng/ml	**Standard**	2.60
G1, G2	Sample [Diluted 1:100]		1.41

Calculated: 100-fold dilution x 8.6 ng/ml = **860** ng/ml



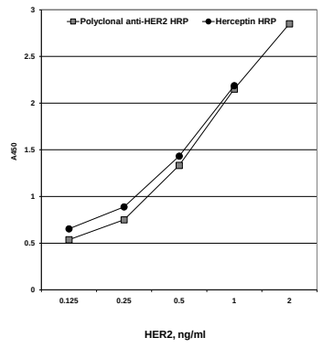
PERFORMANCE CHARACTERISTICS

Specificity

The antibodies used in this kit are specific for the HER2/ErbB2/CD340 protein, recombinant or native, and do not react with other proteins found in serum. The HER2 recombinant protein used as kit standard is recognized by the drug Herceptin with sensitivity equivalent to the Anti-HER2 HRP conjugate of the kit.

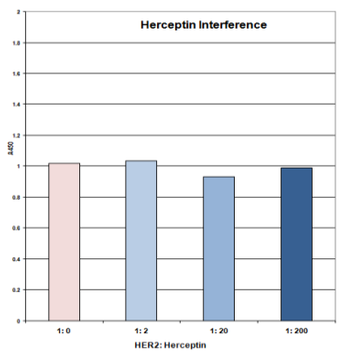
Herceptin

The HER2 Standard shows equivalent dilution curves using either the polyclonal anti-HER2 HRP of the kit or an HRP conjugate of Herceptin.



Interference

Herceptin was mixed with recombinant purified Her2 protein in sample buffer at mass ratios of 1:2 to 1:200, and showed no significant effect on recovery of Her2 when tested in Her2 ELISA:



PERFORMANCE CHARACTERISTICS (cont)

Precision
Samples containing low, medium and high concentrations of HER2 were assayed multiple times in the same assay (n=10) to provide within-assay precision, and as duplicates in multiple assays (n=5) to obtain between-assay reproducibility. Coefficients of variation were calculated for the concentrations using a point-to-point curve-fitting program.

HER2 concentrations were measured with good within-assay (2.5 to 4.1 %CV) and between-assay (4.3 to 9.2 %CV) reproducibility.

Sample	**HER2** ng/ml	**Intra-assay** %CV	**Inter-assay** %CV
High Concn	12.7	**2.5**	**9.2**
Medium Concn	7.6	**2.6**	**4.3**
Low Concn	3.0	**4.1**	**6.8**

LIMITS OF THE ASSAY

1. HER2 that is incomplete in sequence (truncated) or is aggregated and/or associated with other biomolecules may not produce dilution curves **parallel** with the Standard curve. For cases of non-parallelism, it may be useful to establish an alternative Standard curve using the altered HER2 preparation.

2. Recovery of HER2 in human or mouse sera has not been fully established.

STORAGE AND STABILITY

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{o} C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

QUALITY CONTROL

Reagents Accurate and reproducible assay results rely on proper storage, handling and control of reagent and sample temperature. Store all reagents as indicated, and warm to room temperature only those to be used in the assay. Shelf-life of the critical reagents and samples will diminish with extended exposure to non-refrigeration, resulting in inaccurate assay results. All solutions should be clear. Cloudiness or particulates are indications of reagent contamination or instability and may interfere with proper performance of the assay. Do not use.

Standard Curve The signal generated by the standards should be continuously increasing in OD from the lowest Standard to the highest Standard, with a difference greater than 1.2 OD. Non-uniform or low signals may indicate problems with technique, protocol directions and/or reagent preparation, use or stability. Do not rely on results generated from an assay with these issues.

A Sample Diluent blank should also be run; OD should be <0.3 and lower than 1 ng/ml Standard OD.

Equipment Precision of results relies on uniform and effective washing techniques; an automatic washer may be used. ELISA reader and pipettes should be properly calibrated.

Instruction Manual No. M-200-530-HER

Human HER2/ErbB2/CD340

ELISA Kit Cat. No. 200-530-HER

For Quantitation of HER2 protein in Solution

For research use only, not for diagnostic or therapeutic use.

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ELISA Kit Components	**Amount**	**Part**
Anti-HER2 Coated Microwell Strip Plate	8-well strips (12)	200-531
HER2 Standard, Lyophilized	2 vials	200-532
Anti-HER2 HRP Conjugate (100X)	0.15 ml	200-533
Sample Diluent Concentrate (20x)	10 ml	SD20T
Wash Solution Concentrate (100X)	10 ml	WB-100
TMB Substrate	12 ml	80091
Stop Solution	12 ml	80101
Product Manual	1 ea	200-530-HER