Version 22-04/24

User's Manual

Human ERBB2 ELISA Kit

REF **DEIA7395**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

For quantitative detection of human ERBB2 in cell culture supernatants, cell lysates, serum and plasma (heparin, EDTA).

General Description

CD's Human ERBB2 Pre-Coated ELISA (Enzyme-Linked Immunosorbent Assay) kit is a solid-phase immunoassay specially designed to measure Human ERBB2 with a 96-well strip plate that is pre-coated with antibody specific for ERBB2. The detection antibody is a biotinylated antibody specific for ERBB2. The capture antibody is polyclonal antibody from goat and the detection antibody is polyclonal antibody from goat. The kit includes Human ERBB2 protein as standards.

To measure Human ERBB2, add standards and samples to the wells, then add the biotinylated detection antibody. Wash the wells with PBS or TBS buffer, and add Avidin-Biotin-Peroxidase Complex (ABC-HRP). Wash away the unbounded ABC-HRP with PBS or TBS buffer and add TMB. TMB is an HRP substrate and will be catalyzed to produce a blue color product, which changes into yellow after adding the acidic stop solution. The absorbance of the yellow product at 450 nm is linearly proportional to Human ERBB2 in the sample. Read the absorbance of the yellow product in each well using a plate reader, and benchmark the sample wells' readings against the standard curve to determine the concentration of Human ERBB2 in the sample.

Reagents And Materials Provided

1. Anti-Human ERBB2 Pre-coated 96-well Strip Microplate: 1, 12 strips of 8 wells Return unused wells to the foil pouch. Reseal along the entire edge of the zip-seal. May be stored for up to 1 month at 4°C provided this is within the expiration date of the kit.
2. Human ERBB2 Standard: 2, 10 ng/tube. Discard the ERBB2 stock solution after 12 hours at 4°C. May be stored at -20°C for 48 hours.
3. Human ERBB2 Biotinylated Antibody (100x): 1, 100 µl.
4. Avidin-Biotin-Peroxidase Complex (100x): 1 100 µl.
5. Sample Diluent: 1, 30 ml.
6. Antibody Diluent: 1, 12 ml.
7. Avidin-Biotin-Peroxidase Diluent: 1, 12 ml.
8. Color Developing Reagent (TMB): 1, 10 ml.
9. Stop Solution: 1, 10 ml.
10. Wash Buffer (25x): 1, 20 ml.
11. Plate Sealers: 4 Piece.

Materials Required But Not Supplied

1. Microplate reader capable of reading absorbance at 450 nm.

2. Automated plate washer (optional).
3. Pipettes and pipette tips capable of precisely dispensing 0.5 µl through 1 ml volumes of aqueous solutions.
4. Multichannel pipettes are recommended for a large numbers of samples.
5. Deionized or distilled water.
6. 500 ml graduated cylinders.
7. Test tubes for dilution.

Storage

Store at 4°C for 6 months, at -20°C for 12 months. Avoid multiple freeze-thaw cycles (Ships with gel ice, can store for up to 3 days in room temperature.)

Specimen Collection And Preparation

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

1. **Serum:** Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 ×g for 15 min. Analyze the serum immediately or aliquot and store samples at -20°C.
2. **Cell culture supernates:** Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.
3. **Plasma:** Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at 1500 ×g within 30 min of collection.

Assay immediately or aliquot and store samples at -20°C.

Samples The user needs to estimate the concentration of the target protein in the sample and select a proper dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve. Dilute the sample using the provided diluent buffer. The following is a guideline for sample dilution. Several trials may be necessary in practice. The sample must be well mixed with the diluents buffer.

High target protein concentration (40-100 ng/ml). The working dilution is 1:100. i.e. Add 3 µl sample into 297 µl sample diluent buffer.

Medium target protein concentration (4-40 ng/ml). The working dilution is 1:10. i.e. Add 225 µl sample into 25 µl sample diluent buffer.

Low target protein concentration (62.5-4000 pg/ml). The working dilution is 1:2. i.e. Add 100 µl sample to 100 µl sample diluent buffer.

Very Low target protein concentration (≤62.5 pg/ml). No dilution necessary, or the working dilution is 1:2.

Plate Preparation

The included microplate is coated with capture antibodies and is ready-to-use. It does not require additional washing or blocking. The unused well strips should be sealed and stored in the original packaging.

Reagent Preparation

1. All reagents: Bring all reagents to room temperature (18-25°C) prior to use. Please DO NOT equilibrate unused plate well strips to room temperature. They should be sealed and stored in the original packaging. The assay can also be done at room temperature however we recommend doing it at 37°C for best consistency with our QC results. Also, the TMB incubation time estimate (15-25 min) is based on incubation at 37°C.
2. **Wash buffer:** Prepare 500 ml of Working Wash Buffer by diluting the supplied 20 ml of Wash Buffer (25 x) with 480 ml of deionized or distilled water. If crystals have formed in the concentrate, warm to room temperature and mix it gently until crystals have completely dissolved.
3. **Biotinylated Anti-Human ERBB2 antibody:** It is recommended to prepare this reagent immediately prior to use by diluting the Human ERBB2 Biotinylated antibody (100x) 1:100 with Antibody Diluent. Prepare 100 µl by adding 1 µl of Biotinylated antibody (100x) to 99 µl of Antibody Diluent for each well. Mi×gently and thoroughly and use within 2 hours of generation.
4. **Avidin-Biotin-Peroxidase Complex:** It is recommended to prepare this reagent immediately prior to use by diluting the Avidin-Biotin-Peroxidase Complex (100x) 1:100 with Avidin-Biotin-Peroxidase Diluent. Prepare 100 µl by adding 1 µl of Avidin-Biotin Peroxidase Complex (100x) to 99 µl of Avidin-Biotin-Peroxidase Diluent for each well. Mi×gently and thoroughly and use within 2 hours of generation.
5. **Human ERBB2 Standard:** It is recommended that the standards be prepared no more than 2 hours prior to performing the experiment. Use one 10 ng of lyophilized Human ERBB2 standard for each experiment. Gently spin the vial prior to use. Reconstitute the standard to a stock concentration of 10 ng/ml using 1 ml of sample diluent. Allow the standard to sit for a minimum of 10 minutes with gentle agitation prior to making dilutions. Use one tube for each experiment.

   a. 10000 pg/ml of Human ERBB2 standard solution: Add 1 ml sample diluent buffer into one tube, keep the tube at room temperature for 10 min and mix thoroughly.

   b. 4000 pg/ml of Human ERBB2 standard solution: Add 0.4 ml of the above ERBB2 standard solution into 0.6 ml sample diluent buffer and mix thoroughly.

   c. 2000 pg/ml→62.5 pg/ml of Human ERBB2 standard solutions: Label 6 Eppendorf tubes with 2000 pg/ml, 1000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml respectively. Aliquot 0.3 ml of the sample diluent buffer into each tube. Add 0.3 ml of the above 4000 pg/ml ERBB2 standard solution into 1st tube and mix. Transfer 0.3ml from 1st tube to 2nd tube and mix. Transfer 0.3 ml from 2nd tube to 3rd tube and mix, and so on.

Assay Procedure

Preparations Before Assay. Please read the following instructions before starting the experiment.

1. Read this manual in its entirety in order to minimize the chance of error.
2. Confirm that you have the appropriate non-supplied equipment available.
3. Confirm that the species, target antigen, and sensitivity of this kit are appropriate for your intended application.
4. Confirm that your samples have been prepared appropriately based upon recommendations (see Sample Preparation) and that you have sufficient sample volume for use in the assay.
5. When first using a kit, appropriate validation steps should be taken before using valuable samples. Confirm

that the kit adequately detects the target antigen in your intended sample type(s) by running control samples.

6. If the concentration of target antigen within your samples is unknown, a preliminary experiment should be run using a control sample to determine the optimal sample dilution (see Sample Preparation).
7. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, a pilot experiment using standards and a small number of samples is recommended.
8. Before using the kit, spin tubes to bring down all components to the bottom of the tubes.
9. Don't let the 96-well plate dry out since this will inactivate active components on the plate.
10. Don't reuse tips and tubes to avoid cross-contamination.
11. Avoid using the reagents from different batches together.
12. The kit should not be used beyond the expiration date on the kit label. 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.

Assay

It is recommended that all reagents and materials be equilibrated to room temperature (18-25°C) prior to the experiment (see Preparation Before The Experiment, if you have missed this information).

1. Prepare all reagents and working standards as directed previously.
2. Remove excess microplate strips from the plate frame and seal and store them in the original packaging.
3. Add 100 µl of the standard, samples, or control per well. Add 100 µl of the Sample Diluent into the zero well. At least two replicates of each standard, sample, or control is recommended.
4. Cover with the plate sealer provided and incubate for 120 minutes at room temperature (or 90 min, 37 °C).
5. Remove the cover and discard the liquid in the wells into an appropriate waste receptacle. Invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.
6. Add 100 µl of the prepared 1x Biotinylated Anti-Human ERBB2 antibody to each well.
7. Cover with a plate sealer and incubate for 90 minutes at room temperature (or 60 minutes at 37°C).
8. Wash the plate 3 times with the 1x wash buffer:

   a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that the wells are not allowed to completely dry at any time.

   b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).

   c. Repeat steps a-b 2 additional times.

   d. Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid.
9. Add 100 µl of the prepared 1x Avidin-Biotin-Peroxidase Complex into each well. Cover with the plate sealer provided and incubate for 40 minutes at RT (or 30 minutes at 37°C).
10. Wash the plate 5 times with the 1x wash buffer:

    a. Discard the liquid in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid. It is recommended that

the wells are not allowed to completely dry at any time.

b. Add 300 µl of the 1x wash buffer to each assay well. (For cleaner background incubate for 60 seconds between each wash).

c. Repeat steps a-b 4 additional times.

d. Discard the wash buffer in the wells into an appropriate waste receptacle. Then, invert the plate on the benchtop onto a paper towel and tap the plate to gently blot any remaining liquid.

11. Add 90 µl of Color Developing Reagent to each well. Cover with the plate sealer provided and incubate in the dark for 30 minutes at RT (or 15-25 minutes at 37°C). (The optimal incubation time must be empirically determined. A guideline to look for is blue shading the top four standard wells, while the remaining standards remain clear.)
12. Add 100 µl of Stop Solution to each well. The color should immediately change to yellow.
13. Within 30 minutes of stopping the reaction, the O.D. absorbance should be read with a microplate reader at 450 nm.

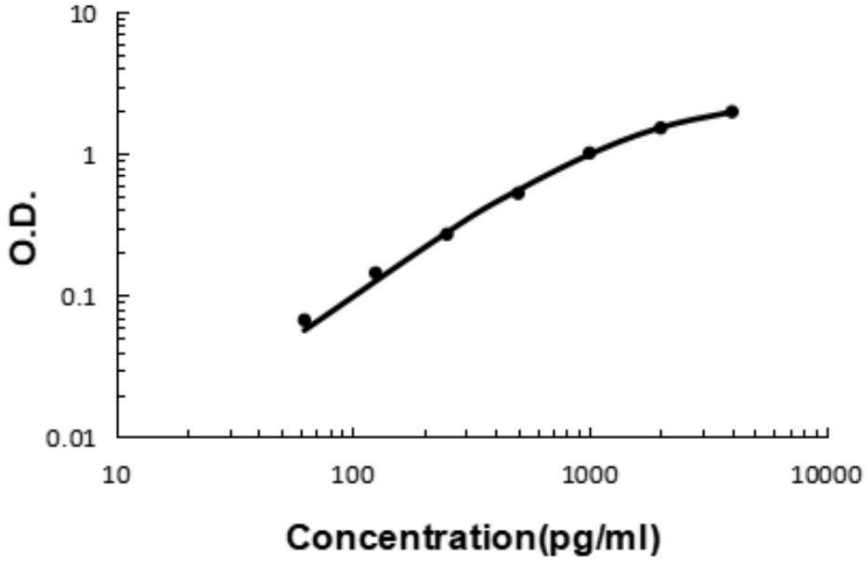
Assay Protocol Notes

1. Solutions: To avoid cross-contamination, change pipette tips between additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
2. Applying Solutions: All solutions should be added to the bottom of the ELISA plate well. Avoid touching the inside wall of the well. Avoid foaming when possible.
3. Assay Timing: The interval between adding samples to the first and last wells should be minimized. Delays will increase the incubation time differential between wells, which will significantly affect the experimental accuracy and repeatability. For each step in the procedure, total dispensing time for addition of reagents or samples should not exceed 10 minutes.
4. Incubation: To prevent evaporation and ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods of time between incubation steps. Do not let wells dry out at any time during the assay. Strictly observe the recommended incubation times and temperatures.
5. Washing: Proper washing procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings. Residual liquid in the reaction wells should be patted dry against absorbent paper during the washing process. Do not put absorbent paper directly into the reaction wells.
6. Controlling Substrate Reaction Time: After the addition of the TMB Substrate, periodically monitor the color development. Stop color development before the color becomes too deep by adding Stop Solution. The excessively strong color will result in inaccurate absorbance readings.
7. Reading: The microplate reader should be preheated and programmed prior to use. Prior to taking O.D. readings, remove any residual liquid or fingerprints from the underside of the plate and confirm that there are no bubbles in the wells.
8. Reaction Time Control: Control reaction time should be strictly followed as outlined.
9. Stop Solution: The Stop Solution contains an acid, therefore proper precautions should be taken during its use, such as protection of the eyes, hands, face, and clothing.
10. To minimize the external influence on the assay performance, operational procedures and lab conditions (such as room temperature, humidity, incubator temperature) should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same operator from the beginning to the end.

Typical Standard Curve

Example (TMB reaction incubate at 37°C):

Concentration (pg/ml)	0	62.5	125	250	500	1000	2000	4000
O.D.	0.025	0.111	0.156	0.286	0.611	0.928	1.368	2.203



Evaluation

It is recommended that a standard curve be created using computer software to generate a four-parameter logistic (4-PL) curve-fit. A free program capable of generating a four-parameter logistic (4-PL) curve-fit can be found online at: www.myassays.com/four-parameter-logistic-curve.assay. Alternatively, plot the mean absorbance for each standard against the concentration. The measured concentration in the sample can be interpolated by using linear regression of each average relative O.D. against the standard curve generated using curve fitting software. This will generate an adequate but less precise fit of the data. For diluted samples, the concentration reading from the standard curve must be multiplied by the dilution factor.

Detection Range

62.5 pg/ml - 4000 pg/ml

Sensitivity

<10 pg/ml

Specificity

Natural and recombinant Human ERBB2.

This kit is for the detection of Human ERBB2. No significant cross-reactivity or interference between ERBB2 and its analogs was observed. This claim is limited by existing techniques therefore cross-reactivity may exist with untested analogs.

Reproducibility

Lots	Lot 1 (pg/ml)	Lot 2 (pg/ml)	Lot 3 (pg/ml)	Lot 4 (pg/ml)	Mean (pg/ml)	Standard Deviation	CV (%)
Sample 1	164	178	168	153	165	8.95	5.4%
Sample 2	544	528	493	539	526	19.91	3.7%
Sample 3	1478	1506	1587	1640	1552	64.34	4.1%

*number of samples for each test n=16.