



User's Manual

Human ERBB2 Matched Antibody Pair

REF

ABPR-C001



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.

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PRODUCT INFORMATION

Intended Use

The Human HER2 / ErbB2 / CD340 ELISA Pair Set is for the quantitative determination of Human HER2 / ErbB2 / CD340.

Principles of Testing

This ELISA Pair Set is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). It utilizes a monoclonal antibody specific for Human HER2 / ErbB2 / CD340 coated on a 96-well plate. Standards and samples are added to the wells, and any Human HER2 / ErbB2 / CD340 present binds to the immobilized antibody. The wells are washed and a horseradish peroxidase conjugated rabbit

anti-Human HER2 / ErbB2 / CD340 monoclonal antibody is then added, producing an antibody-antigenantibody "sandwich". The wells are again washed and TMB substrate solution is loaded, which produces color in proportion to the amount of Human HER2 / ErbB2 / CD340 present in the sample. To end the enzyme reaction, the stop solution is added and absorbances of the microwell are read at 450 nm.

Reagents And Materials Provided

1. Capture Antibody 1 mg/mL of rabbit anti-Human HER2 / ErbB2 / CD340 monoclonal antibody (in PBS, pH 7.4). Dilute to a working concentration of 1 µg/mL in PBS before coating.
2. Detection Antibody 0.1 mg/mL of rabbit anti-Human HER2 / ErbB2 / CD340 monoclonal antibody conjugated to horseradish-peroxidase (HRP) (in PBS, 50 % HRP-Protector, pH 7.4, store at 4°C). Dilute to working concentration of 0.5 µg/mL in detection antibody dilution buffer before use.
3. Standard Each vial contains 110 ng of recombinant Human HER2 / ErbB2 / CD340. Reconstitute with 1 mL detection antibody dilution buffer. After reconstitution, store at -20°C to -80°C in a manual defrost freezer. A seven-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 1500 pg/mL is recommended.

Materials Required But Not Supplied

1. PBS - 136.9 mM NaCl, 10.1 mM Na₂HPO₄, 2.7 mM KCl, 1.8 mM KH₂PO₄, pH 7.4, 0.2 µm filtered
2. TBS - 20 mM Tris, 150 mM NaCl, pH 7.4
3. Wash Buffer - 0.05% Tween20 in TBS, pH 7.2 - 7.4
4. Blocking Buffer - 2% BSA in Wash Buffer
5. Sample dilution buffer - 0.1% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered
6. Detection antibody dilution buffer - 0.5% BSA in wash buffer, pH 7.2 - 7.4, 0.2 µm filtered
7. Substrate Solution : To achieve best assay results, fresh substrate solution is recommended
8. Substrate stock solution - 10mg / ml TMB (Tetramethylbenzidine) in DMSO
9. Substrate dilution buffer - 0.05M Na₂HPO₄ and 0.025M citric acid; adjust pH to 5.5
10. Substrate working solution - For each plate dilute 250 µl substrate stock solution in 25ml substrate dilution

buffer and then add 80 µl 0.75% H₂O₂, mix it well

11. Stop Solution - 2 NH₂SO₄

Storage

Capture Antibody: Aliquot and store at -20°C to -80°C for up to 6 months from date of receipt. Avoid repeated freeze-thaw cycles.

Detection Antibody: Store at 4°C and protect it from prolonged exposure to light for up to 6 months from date of receipt. DO NOT FREEZE!

Standard: Store lyophilized standard at -20°C to -80°C for up to 6 months from date of receipt. Aliquot and store the reconstituted Standard at -80°C for up to 1 month. Avoid repeated freeze-thaw cycles.

Plate Preparation

1. Dilute the capture antibody to the working concentration in PBS. Immediately coat a 96-well microplate with 100µL per well of the diluted capture antibody. Seal the plate and incubate overnight at 4°C.
2. Aspirate each well and wash with at least 300µl wash buffer, repeating the process two times for a total of three washes. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining wash buffer by inverting the plate and blotting it against clean paper towels.
3. Block plates by adding 300 µL of blocking buffer to each well. Incubate at room temperature for a minimum of 1 hour.
4. Repeat the aspiration/wash as in step 2. The plates are now ready for sample addition.

Assay Procedure

1. Add 100 µL of sample or standards in sample dilution buffer per well. Seal the plate and incubate 2 hours at room temperature.
2. Repeat the aspiration/wash as in step 2 of plate preparation.
3. Add 100 µL of the detection antibody, diluted in antibody dilution buffer, to each well. Seal the plate and incubate 1 hour at room temperature.
4. Repeat the aspiration/wash as in step 2 of plate preparation.
5. Add 200 µL of substrate solution to each well. Incubate for 20 minutes at room temperature (if substrate solution is not as requested, the incubation time should be optimized). Avoid placing the plate in direct light.
6. Add 50 µL of stop solution to each well. Gently tap the plate to ensure thorough mixing.
7. Determine the optical density of each well immediately, using a microplate reader set to 450 nm.

Calculation

Calculate the mean absorbance for each set of duplicate standards, controls and samples. Subtract the mean zero standard absorbance from each.

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.

To determine the concentration of the unknowns, find the unknowns' mean absorbance value on the y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the x-axis and read the concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Alternatively, computer-based curve-fitting statistical software may also be employed to calculate the concentration of the sample.

Sensitivity

The minimum detectable dose of Human HER2 / ErbB2 / CD340 was determined to be approximately 24 pg/ml. This is defined as at least three times standard deviations above the mean optical density of 10 replicates of the zero standard.

Specificity

The following factors prepared at 1250 ng/mL were assayed and exhibited no cross-reactivity with

Human EGFR/ErbB1/HER1

Human ErbB3/HER3

Human ErbB4/HER4

