AffinityImmuno®

Trastuzumab (Herceptin®) Immunogenicity ELISA

Catalog …

For the qualitative determination of Trastuzumab binding antibodies in serum and plasma.

Trastuzumab (Herceptin®) is a humanized recombinant monoclonal antibody used for the treatment of primary breast cancers overexpressing human epidermal growth factor 2 (HER2). This kit allows researchers to qualitatively monitor the presence of anti-Trastuzumab antibodies in biological matrices.

Principle of the assay

This immunogenicity assay employs the bridging ELISA … technique. Capture antibody is precoated onto … microplate. Qualitative con… into the appropriate wells. Anti-Trastuzumab present in biological matrices is bound by the immobilized capture antibody. After washing away any unbound substances, … secondary antibody is added to the wells and after a final wash a detection reagent is added. The plate is washed to remove any unbound antibody-enzyme reagent and a substrate solution is added to the wells for color development. The color development is proportional to the amount of anti-Trastuzumab present in test samples. Three levels of QC samples give signal which can be used to determine the level of anti-Trastuzumab antibody in the unknown samples. The color development is stopped and the intensity of the color is measured.

Each kit includes	
Coated microtiter plate, 96 wells (1x8 strips)	1
QC samples	[illegible]
[illegible]X wash buffer	25ml
Assay buffer	50ml
1000X secondary antibody	[illegible]
1000X detection reagent	[illegible]
TMB	12ml
TMB stop solution	12ml
Plate sealers	3

Do not mix or substitute reagents with those from other lots.

Materials and instruments not supplied

- Precision pipettes cal…
- Multichannel pipette…
- Plate …
- Disposable tips
- Vortex mixer
- Distilled or deionize…
- Microplate reader capa… background subtraction…

Safety precautions

- The test protocol must be followed strictly.
- All reagents contain… be handled as if potentio… should wear gloves and protective clothing when handling any patient sera or serum based products.
- The kit reagents contain… and… with the skin and eyes… plenty of water if any contact occurs.
- Any liquid that has be… potentially infectious material has to be discarded in a container with a… performed in accordance with local regulations.
- Disposal must be perfor… local regulations.
- Only trained laborator… this test.

[redacted]	
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[redacted]	

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Preparation of reagents

Prepare only the appropriate amount of required reagent on the day of use. Store all reagents as per instructions stated on the label.

1. **Wash Buffer (1X) Preparation:** Dilute wash buffer concentrate with ultra-pure water 1/10 before use (for example add 2mL concentrate to 18mL ultra-pure water). Mix well.
2. **Secondary antibody (1X) Preparation:** Dilute secondary antibody with assay buffer 1/1000 before use (for examples add 12µl concentrate to 12ml of assay buffer). Mix well.
3. **Detection Reagent (1X) Preparation:** Dilute detection reagent with assay buffer 1/1000 before use (for example add 12µl concentrate to 12ml of assay buffer). Mix well.

Specimen storage

This kit is compatible with EDTA-plasma, heparin-plasma and serum samples. Samples can be stored at or below -20°C for up to 1 year.

Assay procedure

1. Remove coated microtiter plate from -20ºC and allow it to acclimate to room temperature for 15-20 minutes.
2. Dilute QC samples and test samples 1/10 with assay buffer (for example add 30µL of prepared calibrator or sample to 270µL of assay buffer). Mix well. Do not store diluted samples.
3. Add 100µL diluted calibrators and samples to appropriate wells on the plate. Incubate for 1 hour at room temperature on a plate shaker at approximately 300rpm.
4. Discard the content of the plate and wash the wells 3x with 200µL wash buffer per well.
5. Add 100µL secondary antibody to appropriate wells on the plate. Incubate for 1 hour at room temperature on a plate shaker at approx 300rpm.
6. Discard the content of the plate and wash the wells 3x with 200µL wash buffer per well.
7. Add 100µL detection reagent to appropriate wells on the plate. Incubate for 1 hour at room temperature on a plate shaker at approx 300rpm.
8. Discard the content of the plate and wash the wells 3x with 200µL wash buffer per well.
9. Add 100µL of TMB to each well on plate. Incubate for 6-10 minutes at room temperature protected from light.
10. Add 100µL of TMB stop solution to each well on plate. Mix by gently tapping the side of the plate.
11. Determine absorbance with a microplate reader at 450nm against 620nm.

Calculations and results

1. Because anti-drug antibodies will vary in terms of affinity and concentration, this assay provides a qualitative readout. As such the user should use the comparable positive controls when comparing interassay results. The provided controls are tested for comparability between lots and can be traced.
2. The anti-drug antibody titers in the test samples will fall in the range of high, medium, low or negative. We recommend each lab develop their own statistical cutpoint using methodologies as described by G. Shankar, et al. (2008). (Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. J. Pharmaceutical and Biomedical Analysis 48:1267–1281).
3. Any sample undiluted or diluted still reading greater than the highest standard should be diluted appropriately with assay buffer and retested. If the samples have been diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

Performance characteristics

Precision: The precision was determined by analyzing samples spiked at 500ng/mL anti-drug antibody in 6 replicates on 6 different occasions. Intra-assay coefficient of variation (CV) ranges from 3% to 14%. Inter-assay CV was at 12%.

Ordering Information

Please vist www.affinityimmuno.com to order this product. Visa, Mastercard, AMEX and PayPal are accepted in our online store.

Your order will be processed immediately and you will be notified with a delivery timeframe.

Materials and storage

Store kit components at -20°C unless specified otherwise. DO NOT USE past kit expiration date. Some vials contain a small amount of reagents. Spin tubes on pulse setting prior to opening.as