

Pharmacokinetic (PK) ELISA Antigen Capture Format

PK Antigen Capture Format - Trastuzumab Drug/Target Complex

For use with anti-trastuzumab monoclonal antibody product HCA263

This method provides a procedure for carrying out a PK ELISA Antigen Capture Format with anti-trastuzumab antibody, product code HCA263 (detection antibody), and using trastuzumab monoclonal antibody for the standard curve. Anti-trastuzumab drug/target complex antibody recognizes trastuzumab only when bound to its target human ErbB2. It does not recognize the free drug or unbound human ErbB2. The method should always be used in conjunction with product and batch specific information provided with each vial (see product datasheets). This protocol will need to be adjusted for use with different detection methods and immunoassay technology platforms.

Reagents

- BSA
- HISPEC immunoassay diluent (BUF049)
- Mouse anti-Penta Histidine Tag:HRP (MCA5995P)
- Human Serum (Sigma-Aldrich, H4522)
- Lynx Rapid HRP Antibody Conjugation Kit® (LNK001P-LNK006P)

For best results when conjugating with Lynx Rapid HRP Antibody Conjugation Kit, avoid using antibody with thiomersal as preservative. Contact us to discuss thiomersal-free options.

- PBS
 - 136 mM NaCl
 - 2.68 mM KCl
 - 8.1 mM Na₂HPO₄
 - 1.46 mM KH₂PO₄
- PBST
 - PBS with 0.05% Tween®-20
- QuantaBlu™ fluorogenic peroxidase substrate (Thermo Fisher Scientific, 15169)

Materials

- 384-well microtiter plate, black, square flat-bottom wells, MaxiSorp™ PS (Thermo Fisher Scientific, 460518)
- Fluorescence plate reader

96-well plates can be used instead of 384-well plates, e.g. black, flat-bottom MaxiSorp PS (Thermo Fisher Scientific, 437111). For the 96-well format, use 100 µl (instead of 20 µl) of antigen, antibodies, or substrate and 300 µl for the blocking step.

1. Prepare human ErbB2 (capture antigen) at 5 µg/ml in PBS. Coat the required number of wells of a 384-well microtiter plate with 20 µl per well of the prepared capture antigen. Incubate overnight at 4°C.
2. Wash the microtiter plate five times with PBST.
3. Block the microtiter plate by adding 100 µl 5% BSA in PBST to each well, and then incubate for 1 hour at room temperature (RT).
4. Wash the microtiter plate five times with PBST.
5. For the standard curve, prepare a dilution series of trastuzumab in 10% human serum in PBST in triplicate. Final concentrations of trastuzumab should cover the range from 0.1 ng/ml to 1,000 ng/ml. Include a zero trastuzumab concentration as the background value.
6. Add 20 µl of each of the diluted standards to the wells designated for the standard curve (in triplicate for each standard recommended). Add 20 µl of each test sample to the other wells (in triplicate for each sample recommended). Incubate for 1 hour at RT.
7. Wash the microtiter plate five times with PBST.
8. To each well, add 20 µl human anti-trastuzumab antibody HCA263 (AbD25279) at a concentration of 2 µg/ml in PBST buffer. Incubate for 1 hour at RT.
9. Wash the microtiter plate five times with PBST.
10. To each well, add 20 µl anti-Penta Histidine Tag:HRP (MCA5995P) at a 1:2000 dilution in HISPEC buffer. Incubate for 1 hour at RT.
11. Wash the microtiter plate ten times with PBST.
12. Add 20 µl QuantaBlu to each well and measure the fluorescence after 30 minutes.

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