Protocol

Pharmacokinetic (PK) ELISA Antigen-Capture Format

PK Antigen Capture Format – Golimumab Drug/Target Complex

For use with anti-golimumab drug/target complex monoclonal antibody product HCA245 or HCA274
This method provides a procedure for carrying out a PK ELISA Antigen Capture Format with anti-golimumab antibodies, product codes HCA245 or HCA274 (detection antibody), and using golimumab monoclonal antibody for the standard curve. HCA274 is an affinity matured antibody that enables development of a higher sensitivity ELISA. Anti-golimumab drug/target complex antibody recognizes golimumab only when bound to its target TNFα. It does not recognize the free drug or unbound TNFα. 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)
- 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 Na_2HPO_4
  - 1.46 mM KH_2PO_4
- 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.

Method

1. Prepare detection antibody: conjugate the antibody HCA274 (AbD25705, monovalent Fab) or HCA245 (AbD20893_hIgG1) using a Lynx Rapid HRP Antibody Conjugation Kit.
2. Prepare the human capture antigen TNFα 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.
3. Wash the microtiter plate five times with PBST.
4. 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).
5. Wash the microtiter plate five times with PBST.
6. For the standard curve, prepare a dilution series of golimumab in 10% human serum in PBST in triplicate. Final concentrations of golimumab should cover the range from 0.1 ng/ml to 1,000 ng/ml. Include a zero golimumab concentration as the background value.
7. 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.
8. Wash the microtiter plate five times with PBST.
9. To each well, add 20 µl pre-prepared HRP conjugated detection antibody at 2 µg/ml in HISPEC buffer. Incubate for 1 hour at RT.
10. Wash the microtiter plate ten times with PBST.
11. Add 20 µl QuantaBlu to each well and measure the fluorescence after 30 minutes.

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