

Pharmacokinetic (PK) Bridging ELISA

PK – Etanercept

For use with anti-etanercept monoclonal antibody products HCA276 and HCA279P

This method provides a procedure for carrying out a PK ELISA with anti-etanercept antibodies, catalog numbers HCA276 (capture antibody) and HCA279P (detection antibody), and using etanercept for the standard curve. HCA279P anti-etanercept antibody does not inhibit the binding of etanercept to TNF α and therefore this assay can detect total drug (free, partially bound and fully bound). 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)
- 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 μ l (instead of 20 μ l) of antigen, antibodies or substrate, and 300 μ l for the blocking step.

Method

1. Prepare the anti-etanercept capture antibody HCA276 (AbD25939) at 5 μ g/ml in PBS. Coat the required number of wells of a 384-well microtiter plate with 20 μ l per well of the prepared capture antibody, and incubate overnight at 4°C.
2. Wash the microtiter five times with PBST.

3. Block the microtiter plate by adding 100 μ l 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 the etanercept in 10% human serum in PBST in triplicate. Final concentration of etanercept should cover the range from 0.1 ng/ml to 1,000 ng/ml. Include a zero etanercept concentration as the background value.
6. Add 20 μ l of each of the diluted standards to the wells designated for the standard curve (in triplicate for each standard recommended). Add 20 μ l 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 μ l HRP conjugated anti-etanercept detection antibody HCA279P (AbD26183_hlgG1) diluted to 2 μ g/ml in HISPEC buffer and incubate for 1 hour at RT.
9. Wash the microtiter plate ten times with PBST.
10. Add 20 μ l QuantaBlu to each well and measure the fluorescence after 30 minutes.

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V1.2.2017