Protocol

Pharmacokinetic (PK) Bridging ELISA

PK - Belatacept

For use with Anti-Belatacept Monoclonal Antibodies catalog #HCA391 and #HCA393
This method provides a procedure for carrying out a PK ELISA with Anti-Belatacept Antibodies, #HCA391 (capture antibody), and #HCA393 (detection antibody), and using belatacept for the standard curve. 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 (Sigma-Aldrich, #A7906)
- HISPEC Assay Diluent (#BUF049)
- Human Serum (Sigma-Aldrich, #H4522)
- LYNX Rapid HRP Antibody Conjugation Kit™ (#LNK001P-LNK006P)
- 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 (Merck Millipore, #817072)
- QuantaBlu Fluorogenic Peroxidase Substrate (Thermo Fisher Scientific, #15169)
- Belatacept

Materials

- 384-well microtiter plate (black, square flat-bottom wells) for example, Black 384-Well Immuno Plates (Thermo Fisher Scientific, #460518)
- Fluorescence plate reader

96-well plates can be used instead of 384-well plates (black, flat-bottom wells) for example, Black 96-Well Immuno Plates (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 the detection Anti-Belatacept Antibody: conjugate #HCA393 (AbD36935ia) using a LYNX Rapid HRP Antibody Conjugation Kit
2. Prepare the capture Anti-Belatacept Antibody #HCA391 (AbD37060) at 1 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 antibody, and incubate overnight at 4°C.
3. Wash the microtiter plate five times (5x) with PBST.
4. Block the microtiter plate by adding 100 µl 5% BSA in PBST to each well, and then incubate for 1 hr at RT.
5. Wash the microtiter plate 5x with PBST.
6. For the standard curve, prepare a dilution series of belatacept in 10% human serum in PBST in triplicate. Final concentration of belatacept should cover the range from 0.01 ng/ml to 4,000 ng/ml. Include a zero belatacept concentration as the background value.
7. Add 20 µl of belatacept dilution per well (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 hr at RT.
8. Wash the microtiter plate 5x with PBST.
9. To each well, add 20 µl HRP conjugated detection Anti-Belatacept Antibody, #HCA393 (AbD36935ia), at 2 µg/ml in HISPEC Assay Diluent. Incubate for 1 hr at RT.
10. Wash the microtiter plate 10x with PBST.
11. Add 20 µl QuantaBlu Fluorogenic Peroxidase Substrate to each well and measure the fluorescence after 30 min.

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Bulletin 3567 Ver A US/EG *0823 Sig 0123*