Protocol

Anti-Drug Antibody (ADA) Bridging ELISA

ADA – Belatacept

For use with Anti-Belatacept Monoclonal Antibodies catalog #HCA392, #HCA393, and Anti-Abatacept Antibody #HCA336

This method provides a procedure for generating an ADA ELISA standard curve with Anti-Belatacept Antibodies #HCA392, #HCA393, and Anti-Abatacept Antibody #HCA336, using belatacept for capture and detection. Belatacept is a fusion protein that only differs from abatacept by two amino acids, hence the Anti-Abatacept Antibody #HCA336 recognizes both abatacept and belatacept. Belatacept is a recombinant, fusion protein drug consisting of the extracellular domain of human cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4, also known as CD152), linked to a modified Fc portion of human immunoglobulin G1 (hIgG1). 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 antibody: conjugate belatacept using a LYNX Rapid HRP Antibody Conjugation Kit.
2. Prepare the unconjugated belatacept capture antibody 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 an Anti-Belatacept Antibody #HCA392 (AbD37060ia), #HCA393 (AbD36935ia), or Anti-Abatacept Antibody #HCA336 (AbD37065ia) in 10% human serum in PBST in triplicate. Final concentration of anti-belatacept antibody or anti-abatacept antibody should cover the range from 0.1 ng/ml to 60,000 ng/ml. Include a zero anti-belatacept antibody concentration as the background value.
7. Add 20 µl of anti-belatacept antibody or anti-abatacept antibody dilution per well (in triplicate for each standard recommended) and incubate for 1 hr at RT.
8. Wash the microtiter plate 5x with PBST.
9. To each well, add 20 µl HRP conjugated belatacept diluted to 2 µg/ml in HISPEC Assay Diluent and 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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