Protocol

Anti-Drug Antibody (ADA) Bridging ELISA

ADA – Abatacept

For use with Anti-Abatacept Monoclonal Antibodies, catalog #HCA335 or #HCA336, or Mouse Anti-Human CTLA-4 Monoclonal Antibody #MCA1724

Abatacept 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). This method provides a procedure for generating an ADA ELISA standard curve with Anti-Abatacept Antibody #HCA335 or #HCA336, or Anti-Human CTLA-4 Antibody #MCA1724, using abatacept for capture and detection. 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 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
- QuantaBlu Fluorogenic Peroxidase Substrate (Thermo Fisher Scientific, #15169)

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 detection antibody: conjugate abatacept using a LYNX Rapid HRP Antibody Conjugation Kit.
2. Prepare the unconjugated abatacept capture reagent 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-Abatacept Antibody #HCA335 (AbD37058ia), #HCA336 (AbD37065ia), or Mouse Anti-Human CTLA-4 Antibody #MCA1724, in 10% human serum in PBST in triplicate. Final concentration of anti-abatacept or anti-CTLA-4 antibody should cover the range from 0.1 ng/ml to 10,000 ng/ml. Include a zero anti-abatacept or anti-CTLA-4 antibody concentration as the background value.
7. Add 20 µl of anti-abatacept or anti-CTLA-4 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 abatacept 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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