Protocol

Anti-Drug Antibody (ADA) Bridging ELISA

ADA – Etanercept

For use with anti-etanercept monoclonal antibody product HCA277
This method provides a procedure for generating an ADA ELISA standard curve with anti-etanercept antibody, catalog number HCA277, using etanercept 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 immunoassay 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, 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 etanercept using a LYNX Rapid HRP Antibody Conjugation Kit.
2. Prepare unconjugated etanercept 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 etanercept solution, and 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 the anti-etanercept antibody HCA277 (clone AbD25940_hIgG4Pro) in 10% human serum in PBST in triplicate. Final concentration of anti-etanercept antibody should cover the range from 1 ng/ml to 32,000 ng/ml. Include a zero anti-etanercept concentration as the background value.
7. Add 20 µl of anti-etanercept antibody dilution per well (in triplicate for each standard recommended) and incubate for 1 hour at RT.
8. Wash the microtiter plate five times with PBST.
9. To each well, add 20 µl HRP conjugated etanercept diluted to 2 µg/ml in HISPEC buffer and 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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