Protocol

Anti-Drug Antibody (ADA) ELISA

ADA IgE isotype - Adalimumab

For use with anti-adalimumab monoclonal antibody product HCA271 IgE isotype

This method provides a procedure for generating an ADA ELISA standard curve with anti-adalimumab antibody in IgE format, product code HCA271, and an anti-human IgE antibody for 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)
- Mouse anti-human IgE antibody, HRP conjugated (0100-0413P)
- 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 adalimumab at 5 µg/ml in PBS. Coat the required number of wells of a 384-well microtiter plate with 20 µl per well of the prepared adalimumab, and incubate overnight at 4°C.
2. Wash the microtiter plate five times with PBST.
3. 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).
4. Wash the microtiter plate five times with PBST.
5. For the standard curve, prepare a dilution series of the anti-adalimumab antibody HCA271 (AbD18655_hIgE) in 10% human serum in PBST in triplicate. Final concentration of anti-adalimumab antibody should cover the range from 0.05 ng/ml to 2,000 ng/ml. Include a zero anti-adalimumab concentration as the background value.
6. Add 20 µl of anti-adalimumab antibody dilution per well (in triplicate for each standard recommended) and incubate for 1 hour at RT.
7. Wash the microtiter plate five times with PBST.
8. To each well add 20 µl HRP labeled mouse anti-human IgE antibody diluted 1:1,000 in HISPEC buffer and incubate for 1 hour at RT.
9. Wash the microtiter plate ten times with PBST.
10. Add 20 µl QuantaBlu to each well and measure the fluorescence after 30 minutes.

V1.11.2016

BIO-RAD