Protocol

Pharmacokinetic (PK) ELISA Antigen Capture Format

PK - Secukinumab

For Use with Anti-Secukinumab Monoclonal Antibody catalog #HCA375

This method provides a procedure for carrying out a PK ELISA antigen capture format with Anti-Secukinumab Antibody, #HCA375 (detection antibody), and using secukinumab for the standard curve. Anti-Secukinumab drug/target complex antibody recognizes secukinumab only when bound to its target human interleukin 17A (IL-17A). It does not recognize the free drug or unbound human IL-17A. 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)
- 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)
- Recombinant Human IL-17A (#PHP294)
- Anti-FLAG M2-Peroxidase (HRP) Antibody (Sigma-Aldrich, #A8592)

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 human IL-17A protein (capture antigen) 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 capture antibody, and incubate overnight at 4°C.
2. Wash the microtiter plate five times (5x) with PBST.
3. Block the microtiter plate by adding 100 µl 5% BSA in PBST to each well, and then incubate for 1 hr at RT.
4. Wash the microtiter plate 5x with PBST.
5. For the standard curve, prepare a dilution series of secukinumab in 10% human serum in PBST in triplicate. Final concentration of secukinumab should cover the range from 0.01 ng/ml to 10,000 ng/ml. Include a zero secukinumab concentration as the background value.
6. Add 20 µl of secukinumab 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.
7. Wash the microtiter plate 5x with PBST.
8. To each well, add 20 µl detection Anti-Secukinumab Antibody, #HCA375 (AbD36949) at 2 µg/ml in PBST. Incubate for 1 hr at RT.
9. Wash the microtiter plate 5x with PBST.
10. To each well, add 20 µl Anti-FLAG M2-Peroxidase (HRP) Antibody at a 1:20,000 dilution in HISPEC Assay Diluent. Incubate for 1 hour at RT.
11. Wash the microtiter plate 10x with PBST.
12. Add 20 µl QuantaBlu Fluorogenic Peroxidase Substrate to each well and measure the fluorescence after 30 min.

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