Protocol

Pharmacokinetic (PK) ELISA Antigen Capture Format

PK

For use with Anti-Human IgG1 (Fc) Monoclonal Antibody catalog #HCA285P
This method provides a procedure for carrying out a PK ELISA Antigen Capture Format with Anti-Human IgG1 (Fc) Antibody, catalog #HCA285P (detection antibody), and using bevacizumab for the standard curve. 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
- Cynomolgus monkey serum (Biotrend, cat. #CYNSRM-5-P-m)
- HISPEC Assay Diluent (cat. #BUF049)
- 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, cat. #15169)
- Recombinant Human VEGF (cat. #PHP091)

Materials

- 384-well microtiter plate, black, square flat-bottom wells, for example, Black 384-Well Immuno Plates (Thermo Fisher Scientific, cat. #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, cat. #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 VEGF (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 antigen, and incubate overnight at 4˚C.
2. Wash the microtiter 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 bevacizumab in 10% cynomolgus monkey serum in PBST in triplicate. Final concentration of bevacizumab should cover the range from 0.125 ng/ml to 8,000 ng/ml. Include a zero bevacizumab concentration as the background value.
6. Add 20 µl of each of the diluted standards to the wells designated for the standard curve (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 HRP conjugated detection antibody HCA285P (AbD27686) at 2 µg/ml in HISPEC Assay Diluent. Incubate for 1 hr at RT.
9. Wash the microtiter plate 10x with PBST.
10. Add 20 µl QuantaBlu Fluorogenic Peroxidase Substrate to each well and measure the fluorescence after 30 min.

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