Pharmacokinetic (PK) ELISA Antigen Capture Format

Protocol

PK - Avelumab

For Use with Anti-Avelumab Monoclonal Antibody Catalog #TZA042

This method provides a procedure for carrying out a PK ELISA Antigen Capture Format with Anti-Avelumab Antibody #TZA042 (detection antibody), and using avelumab for the standard curve. Anti-avelumab drug/target complex antibody recognizes avelumab only when bound to its target human programmed cell death ligand 1 (PD-L1). It does not recognize the free drug or unbound human PD-L1. 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 (Sigma-Aldrich, #A7906)
- 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 (Merck Millipore, #817072)
- QuantaBlu Fluorogenic Peroxidase Substrate (Thermo Fisher Scientific, #15169)
- Recombinant Human PD-L1 (Sino Biologicals, #10084-H08H)
- 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, #460518). 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 PD-L1 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 antigen, 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 avelumab in 10% human serum in PBST in triplicate. Final concentration of avelumab should cover the range from 0.001 ng/ml to 10,000 ng/ml. Include a zero avelumab concentration as the background value.
6. Add 20 µl of avelumab 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-Avelumab Antibody, #TZA042 (AbD42040ad), 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 buffer. Incubate for 1 hr 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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