Pharmacokinetic (PK) Bridging ELISA

Protocol

PK - Avelumab

For Use with Anti-Avelumab Monoclonal Antibodies Catalog #TZA041 and #TZA040P

This method provides a procedure for carrying out a PK ELISA with Anti-Avelumab Antibodies, #TZA041 (capture antibody) and HRP-conjugated #TZA040P (detection antibody), and using avelumab 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 (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)

Materials:

- 384-well microtiter plate, black, square flat-bottom wells, for example, Black 384-Well Immuno Plates (Thermo Fisher Scientific, #460518) HISPEC Assay Diluent (#BUF049)
- 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 the capture Anti-Avelumab Antibody #TZA041 (AbD40521ad) 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 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 avelumab in 10% human serum in PBST in triplicate. Final concentration of avelumab should cover the range from 0.01 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 HRP conjugated detection Anti-Avelumab Antibody, #TZA040P (AbD40519pap), at 0.5 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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