

Pharmacokinetic (PK) Bridging ELISA

PK – Bevacizumab

For use with anti-bevacizumab monoclonal antibody products HCA182 and HCA184P

This method provides a procedure for carrying out a PK ELISA with anti-bevacizumab antibodies, product codes HCA182 (capture antibody) and HCA184P (detection antibody), and using bevacizumab monoclonal antibody 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
- HISPEC immunoassay diluent (BUF049)
- Human Serum (Sigma-Aldrich, H4522)
- PBS
 - 136 mM NaCl
 - 2.68 mM KCl
 - 8.1 mM Na₂HPO₄
 - 1.46 mM KH₂PO₄
- 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 the anti-bevacizumab capture antibody HCA182 (AbD17976) 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. Incubate overnight at 4°C.
2. Wash the microtiter 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 bevacizumab in 10% human serum in PBST in triplicate. Final concentrations of bevacizumab should cover the range from 0.1 ng/ml to 1,000 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 hour at RT.
7. Wash the microtiter plate five times with PBST.
8. To each well, add 20 µl HRP conjugated detection antibody HCA184P (AbD16748_hlgG1) at 4 µg/ml in HISPEC buffer. 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.

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