Protocol

Pharmacokinetic (PK) Bridging ELISA

PK – Cetuximab

For Use with Anti-Cetuximab Monoclonal Antibodies catalog #HCA220 and #HCA228P

This method provides a procedure for carrying out a PK ELISA with Anti-Cetuximab Antibodies, #HCA220 (capture antibody) and #HCA228P (detection antibody), and using cetuximab for the standard curve. Anti-Cetuximab Antibody #HCA220 is an inhibitory antibody. Anti-Cetuximab Antibody #HCA228P is a non-inhibitory antibody that does not inhibit the binding of cetuximab to its target epidermal growth factor receptor (EGFR). This combination enables measurement of free and partially bound cetuximab, which is equivalent to total drug when trough serum levels are above 4 µg/ml.
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)

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 the capture Anti-Cetuximab Antibody #HCA220 (AbD19834) 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. 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 cetuximab in 10% human serum in PBST in triplicate. Final concentrations of cetuximab should cover the range from 0.1 ng/ml to 1,000 ng/ml. Include a zero cetuximab 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 Anti-Cetuximab Antibody #HCA228P (AbD19376_hIgG1) 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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