

## Pharmacokinetic (PK) Bridging ELISA

### PK – Panitumumab

#### For use with anti-panitumumab monoclonal antibody products HCA264 and HCA265P

This method provides a procedure for carrying out a PK ELISA with anti-panitumumab antibodies, product codes HCA264 (capture antibody) and HCA265P (detection antibody), and using panitumumab 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 Na<sub>2</sub>HPO<sub>4</sub>
  - 1.46 mM KH<sub>2</sub>PO<sub>4</sub>
- 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-panitumumab capture antibody HCA264 (AbD23894) 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 plate 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 panitumumab in 10% human serum in PBST in triplicate. Final concentrations of panitumumab should cover the range from 0.1 ng/ml to 1,000 ng/ml. Include a zero panitumumab concentration as the background.
6. Add 20 µl of each of the diluted standards to the

- 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 HCA265P (AbD23897\_hlgG1) at 2 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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