Pharmacokinetic (PK) Bridging ELISA

PK - Trastuzumab

For use with anti-trastuzumab monoclonal antibody products HCA169 and HCA270
This method provides a procedure for carrying out a PK ELISA with anti-trastuzumab antibodies, product codes HCA169 (capture antibody), and HCA270 (detection antibody, IgG4 isotype) using trastuzumab for the standard curve and an HRP conjugated anti-human IgG4 antibody (MCA2098P) for detection. 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)
- Mouse anti-human IgG4:HRP (MCA2098P)
- 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-trastuzumab capture antibody HCA169 (AbD18141) 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 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 trastuzumab in 10% human serum in PBST in triplicate. Final concentration of trastuzumab should cover the range from 0.125 ng/ml to 8,000 ng/ml. Include a zero trastuzumab 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 detection antibody HCA270 (AbD18018_hlg4_Pro) at 2 µg/ml in PBST. Incubate for 1 hour at RT.
9. Wash the microtiter plate ten times with PBST.
10. To each well add 20 µl HRP conjugated mouse anti-human IgG4 antibody diluted 1:5,000 in HISPEC buffer and incubate for 1 hour at RT.
11. Wash the microtiter plate ten times with PBST.
12. Add 20 µl QuantaBlu to each well and measure the fluorescence after 30 minutes.

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V1.12.2016