

## Pharmacokinetic (PK) ELISA Antigen Capture Format

### PK - Infliximab

#### For use with Anti-Infliximab Monoclonal Antibody catalog #TZA009P

This method provides a procedure for carrying out a PK ELISA Antigen Capture Format with Anti-infliximab Antibody, #TZA009P (detection antibody), and using infliximab for the standard curve. Anti-infliximab drug/target complex antibody recognizes infliximab only when bound to its target human TNF $\alpha$ . It does not recognize the free drug or unbound human TNF $\alpha$ . 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, #A7906-100)
- HISPEC Assay 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 (Merck Millipore, #817072)
- QuantaBlu Fluorogenic Peroxidase Substrate (Thermo Fisher Scientific, #15169)
- Recombinant Human TNF $\alpha$  Protein (Bio-Rad, #PHP051H)
- Human Anti-TNF $\alpha$  Antibody (Bio-Rad, #MCA6090)

#### Materials

- 384-well microtiter plate, black, 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  $\mu$ l (instead of 20  $\mu$ l) of antigen, antibodies, or substrate and 300  $\mu$ l for the blocking step.

#### Method

1. Prepare human TNF $\alpha$  protein (capture antigen) at 5  $\mu$ g/ml in PBS. Coat the required number of wells of a 384-well microtiter plate with 20  $\mu$ l per well of the prepared capture antigen, and incubate overnight at 4°C.
2. Wash the microtiter five times (5x) with PBST.
3. Block the microtiter plate by adding 100  $\mu$ l 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 infliximab in 10% human serum in PBST in triplicate. Final concentration of infliximab should cover the range from 0.001 ng/ml to 1,000 ng/ml. Include a zero infliximab concentration as the background value.
6. Add 20  $\mu$ l of infliximab dilution per well (in triplicate for each standard is recommended). Add 20  $\mu$ l of each test sample to the other wells (in triplicate for each sample is recommended). Incubate for 1 hr at RT.
7. Wash the microtiter plate 5x with PBST.
8. To each well, add 20  $\mu$ l Anti-Infliximab Antibody, #TZA009P (AbD18391pap) at 0.25  $\mu$ g/ml in HISPEC diluent (#BUF049). Incubate for 1 hr at RT.
9. Wash the microtiter plate 10x with PBST.
10. Add 20  $\mu$ l QuantaBlu Fluorogenic Peroxidase Substrate to each well and measure the fluorescence after 30 min.

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