

# Pharmacokinetic (PK) ELISA Antigen Capture Format

## Protocol

### PK - Canakinumab

#### For Use with Anti-Canakinumab Monoclonal Antibody Catalog #TZA0112

This method provides a procedure for carrying out a PK ELISA antigen capture format with Anti-Canakinumab Antibody, #TZA0112 (detection antibody), and using canakinumab for the standard curve. Anti-canakinumab drug/target complex antibody recognizes canakinumab only when bound to its target human interleukin-1 beta (IL-1 $\beta$ ). It does not recognize the free drug or unbound human IL-1 $\beta$ . 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)
- 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
- QuantaBlu Fluorogenic Peroxidase Substrate (Thermo Fisher Scientific, #15169)
- Recombinant Human IL-1 Beta (Sino Biologicals, #10139-HNAE)
- Anti-FLAG M2-Peroxidase (HRP) Antibody (Sigma-Aldrich, #A8592)

### 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  $\mu$ L (instead of 20  $\mu$ L) of antigen, antibodies, or substrate and 300  $\mu$ L for the blocking step.

### Method:

1. Prepare human IL-1 $\beta$  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 plate 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 canakinumab in 10% human serum in PBST in triplicate. Final concentration of canakinumab should cover the range from 0.01 ng/mL to 10,000 ng/mL. Include a zero canakinumab concentration as the background value.
6. Add 20  $\mu$ L of canakinumab dilution per well (in triplicate for each standard recommended). Add 20  $\mu$ L 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  $\mu$ L detection Anti-Canakinumab Antibody, #TZA0112 (AbD54142ad), at 2  $\mu$ g/mL in PBST. Incubate for 1 hr at RT.
9. Wash the microtiter plate 5x with PBST.
10. To each well, add 20  $\mu$ L Anti-FLAG M2-Peroxidase (HRP) Antibody at a 1:20,000 dilution in HISPEC Assay Diluent. Incubate for 1 hr at RT.
11. Wash the microtiter plate 10x with PBST.
12. Add 20  $\mu$ L QuantaBlu Fluorogenic Peroxidase Substrate to each well and measure the fluorescence after 30 min.

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