

## Pharmacokinetic (PK) ELISA Antigen Capture Format

### PK Antigen Capture Format - Adalimumab Drug/Target Complex

#### For use with anti-adalimumab drug/target complex monoclonal antibody products HCA231 or HCA232P

This method provides a procedure for carrying out a PK ELISA Antigen Capture Format with anti-adalimumab antibodies, product codes HCA231 or HCA232P (detection antibody), and using adalimumab monoclonal antibody for the standard curve. Anti-adalimumab drug/target complex antibody recognizes adalimumab only when bound to its target TNF $\alpha$ . It does not recognize the free drug or unbound 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
- HISPEC immunoassay diluent (BUF049)
- Human Serum (Sigma-Aldrich, H4522)
- Lynx Rapid HRP Antibody Conjugation Kit<sup>®</sup> (LNK001P-LNK006P)

*For best results when conjugating with Lynx Rapid HRP Antibody Conjugation Kit, avoid using antibody with thiomersal as preservative. Contact us to discuss thiomersal-free options.*

- 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<sup>®</sup>-20
- QuantaBlu<sup>™</sup> fluorogenic peroxidase substrate (Thermo Fisher Scientific, 15169)

#### Materials

- 384-well microtiter plate, black, square flat-bottom wells, MaxiSorp<sup>™</sup> 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  $\mu$ l (instead of 20  $\mu$ l) of antigen, antibodies, or substrate and 300  $\mu$ l for the blocking step.*

#### Method

1. Prepare detection antibody: if using HCA231 (AbD20350) for detection, first conjugate the antibody using a Lynx Rapid HRP Antibody Conjugation Kit; HCA232P is already directly labeled with HRP.

2. Prepare human TNF $\alpha$  (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. Incubate overnight at 4°C.
3. Wash the microtiter plate five times with PBST.
4. Block the microtiter plate by adding 100  $\mu$ l 5% BSA in PBST to each well, and then incubate for 1 hour at room temperature (RT).
5. Wash the microtiter plate five times with PBST.
6. For the standard curve, prepare a dilution series of adalimumab in 10% human serum in PBST in triplicate. Final concentrations of adalimumab should cover the range from 0.1 ng/ml to 1,000 ng/ml. Include a zero adalimumab concentration as the background value.
7. Add 20  $\mu$ l of each of the diluted standards to the wells designated for the standard curve (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 hour at RT.
8. Wash the microtiter plate five times with PBST.
9. To each well, add 20  $\mu$ l pre-prepared HRP conjugated detection antibody at 2  $\mu$ g/ml in HISPEC buffer. Incubate for 1 hour at RT.
10. Wash the microtiter plate ten times with PBST.
11. Add 20  $\mu$ l QuantaBlu to each well and measure the fluorescence after 30 minutes.

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Tween<sup>®</sup> is a registered trademark of ICI Americas Inc.

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