Protocol

Anti-Drug Antibody (ADA) Bridging ELISA

ADA - Brentuximab vedotin

For Use with Anti-Brentuximab vedotin Monoclonal Antibodies catalog #HCA350, #HCA351, and #HCA352
This method provides a procedure for generating an ADA ELISA standard curve with Anti-Brentuximab vedotin Antibody, #HCA350, #HCA351, or #HCA352 using brentuximab vedotin antibody for capture and 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 Assay Diluent (#BUF049)
- Human Serum (Sigma-Aldrich, #H4522)
- LYNX Rapid HRP Antibody Conjugation Kit (#LNK001P-LNK006P)
- PBS
  - 136 mM NaCl
  - 2.68 mM KCl
  - 8.1 mM Na_2HPO_4
  - 1.46 mM KH_2PO_4
- 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, 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 µl (instead of 20 µl) of antigen, antibodies, or substrate and 300 µl for the blocking step.

Method

1. Prepare detection antibody: conjugate brentuximab vedotin antibody using a LYNX Rapid HRP Antibody Conjugation Kit.
2. Prepare the unconjugated brentuximab vedotin capture antibody 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 brentuximab vedotin, and incubate overnight at 4°C.
3. Wash the microtiter plate five times (5x) with PBST.
4. Block the microtiter plate by adding 100 µl 5% BSA in PBST to each well, and then incubate for 1 hr at RT.
5. Wash the microtiter plate 5x with PBST.
6. For the standard curve, prepare a dilution series of an Anti-Brentuximab vedotin Antibody #HCA350 (AbD39650ia), or #HCA351 (AbD39655ia), or #HCA352 (AbD39659ia) in 10% human serum in PBST in triplicate. Final concentration of anti-brentuximab vedotin antibody should cover the range from 0.1 ng/ml to 10,000 ng/ml. Include a zero anti-brentuximab vedotin antibody concentration as the background value.
7. Add 20 µl of anti-brentuximab vedotin antibody dilution per well (in triplicate for each standard recommended) and incubate for 1 hr at RT.
8. Wash the microtiter plate 5x with PBST.
9. To each well, add 20 µl HRP conjugated brentuximab vedotin diluted to 2 µg/ml in HISPEC Assay Diluent and incubate for 1 hr at RT.
10. Wash the microtiter plate 10x with PBST.
11. Add 20 µl QuantaBlu Fluorogenic Peroxidase Substrate to each well and measure the fluorescence after 30 min.

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