

Anti-Drug Antibody (ADA) Bridging ELISA

ADA - Bevacizumab

For use with anti-bevacizumab monoclonal antibody product HCA185

This method provides a procedure for generating an ADA ELISA standard curve with anti-bevacizumab antibody, product code HCA185. The method should always be used in conjunction with the 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® (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 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 bevacizumab 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 bevacizumab. 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 the anti-bevacizumab antibody HCA185 (AbD17976_hlgG1) in 10% human serum in PBST in triplicate. Final concentrations of anti-bevacizumab antibody should cover the range from 0.1 ng/ml to 10,000 ng/ml. Include a zero anti-bevacizumab concentration as the background value.
6. Add 20 µl of anti-bevacizumab antibody dilution per well (in triplicate for each standard recommended) and incubate for 1 hour at RT.
7. Wash the microtiter plate five times with PBST.
8. To each well add 20 µl HRP conjugated bevacizumab diluted to 2 µg/ml in HISPEC buffer and incubate for 1 hour at RT.
9. Wash the microtiter plate ten times with PBST.
10. Add 20 µl QuantaBlu to each well and measure the fluorescence after 30 minutes.

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