

Anti-Drug Antibody (ADA) Bridging ELISA

ADA - Denosumab

For use with Anti-Denosumab Monoclonal Antibodies catalog #HCA280, #HCA281, #HCA282

This method provides a procedure for generating an ADA ELISA standard curve with an Anti-Denosumab Antibody, cat. #HCA280, #HCA281 or #HCA282 using denosumab 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 (cat. #BUF049)
- Human Serum (Sigma-Aldrich, cat. #H4522)
- LYNX Rapid HRP Antibody Conjugation Kit® (cat. #LNK001P-LNK006P)
- Phosphate buffered saline (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, cat. #15169)

Materials

- 384-well microtiter plate, black, square flat-bottom wells, for example, Black 384-Well Immuno Plates (Thermo Fisher Scientific, cat. #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, cat. #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 denosumab antibody using a LYNX Rapid HRP Antibody Conjugation Kit.
2. Prepare the unconjugated denosumab 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 capture antibody, 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-Denosumab Antibody cat. #HCA280 (AbD26295_hlgG1), #HCA281 (AbD26296_hlgG1) or #HCA282 (AbD26862_hlgG1) in 10% human serum in PBST in triplicate. Final concentration of denosumab antibody should cover the range from 0.1 ng/ml to 8,000 ng/ml. Include a zero anti-denosumab antibody concentration as the background value.
7. Add 20 µl of anti-denosumab 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 denosumab 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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Tween is a trademark of Croda International Plc.

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