

Anti-Drug Antibody (ADA) Bridging ELISA

Protocol

ADA - Ocrelizumab

For Use with Anti-Ocrelizumab Monoclonal Antibodies Catalog #HCA399, #HCA400, and #HCA401

This method provides a procedure for generating an ADA ELISA standard curve with Anti-Ocrelizumab Antibody, #HCA399, #HCA400, or #HCA401 using ocrelizumab 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 (Sigma-Aldrich, #A7906)
- HISPEC Assay 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 (Merck Millipore, #817072)
- 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, 460518). 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 detection antibody: conjugate ocrelizumab antibody using a LYNX Rapid HRP Antibody Conjugation Kit.
2. Prepare the unconjugated ocrelizumab 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 ocrelizumab, 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 the Anti-Ocrelizumab Antibody #HCA399 (AbD47395ia), #HCA400 (AbD50641ia), or #HCA401 (AbD50989ia) in 10% human serum in PBST in triplicate. Final concentration of anti-ocrelizumab antibody should cover the range from 1 ng/ml to 100,000 ng/ml. Include a zero anti-ocrelizumab antibody concentration as the background value.
7. Add 20 µl of anti-ocrelizumab 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 ocrelizumab 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.

Bio-Rad is a trademark of Bio-Rad Laboratories, Inc. in certain jurisdictions. LYNX Rapid Conjugation Kit is a trademark of Bio-Rad Europe, GmbH in certain jurisdictions. All trademarks used herein are the property of their respective owner.



**Bio-Rad
Laboratories, Inc.**

Life Science
Group

Website bio-rad.com **USA** 1 800 424 6723 **Australia** 61 2 9914 2800 **Austria** 00 800 00 24 67 23 **Belgium** 00 800 00 24 67 23 **Brazil** 4003 0399
Canada 1 905 364 3435 **China** 86 21 6169 8500 **Czech Republic** 00 800 00 24 67 23 **Denmark** 00 800 00 24 67 23 **Finland** 00 800 00 24 67 23
France 00 800 00 24 67 23 **Germany** 00 800 00 24 67 23 **Hong Kong** 852 2789 3300 **Hungary** 00 800 00 24 67 23 **India** 91 124 4029300 **Israel** 0 3 9636050
Italy 00 800 00 24 67 23 **Japan** 81 3 6361 7000 **Korea** 82 080 007 7373 **Luxembourg** 00 800 00 24 67 23 **Mexico** 52 555 488 7670
The Netherlands 00 800 00 24 67 23 **New Zealand** 64 9 415 2280 **Norway** 00 800 00 24 67 23 **Poland** 00 800 00 24 67 23 **Portugal** 00 800 00 24 67 23
Russian Federation 00 800 00 24 67 23 **Singapore** 65 6415 3188 **South Africa** 00 800 00 24 67 23 **Spain** 00 800 00 24 67 23 **Sweden** 00 800 00 24 67 23
Switzerland 00 800 00 24 67 23 **Taiwan** 886 2 2578 7189 **Thailand** 66 2 651 8311 **United Arab Emirates** 36 1 459 6150 **United Kingdom** 00 800 00 24 67 23

