

Immunizations and enzyme-linked immunosorbent assay (ELISA) for antibody isotype determination

CURRENT STATUS: POSTED

Stefan Wirtz

Clemens Neufert

Benno Weigmann

Markus F Neurath

DOI:

10.1038/nprot.2007.4100

SUBJECT AREAS

Biological techniques

KEYWORDS

--- - Mouse - Intestinal inflammation - Crohn's disease - Ulcerative colitis

Abstract

This protocol was used in our *Nature Immunology* paper

Reagents

Keyhole limpet hemocyanin (KLH) and DNP-KLH (Calbiochem-Novabiochem)

Alum (Serva)

Sterile 1 ml syringes and 30 gage needles

96-well MaxiSorp™ ELISA plates (Nunc)

DNP-ovalbumin (Sigma)

PBS + 0.05% Tween 20 (Sigma)

PBS + 2.5% bovine serum albumin (Sigma)

Alkaline phosphatase-labeled mouse anti-IgM and subclass IgG-specific antibodies (Southern Biotech Associates)

Alkaline phosphatase substrate solution (freshly made; Bio-Rad)

Procedure

Immunizations of mice

1 Perform precipitation of keyhole limpet hemocyanin (KLH) or DNP-KLH in alum as described (2).

2 Inject 100 µg of KLH or DNP-KLH precipitated in alum in a total volume of 300 µl (volume adjusted with PBS) into the peritoneal cavity. Collect serum at d 0, d 7, and d 14 and make serial dilutions in PBS.

ELISA for antibody isotype determination

3 Coat 96-well MaxiSorp™ ELISA plates with 50 µl/well of DNP-ovalbumin (5 µg/ml in PBS), incubate overnight at 4 °C (3).

4 Wash with PBS + 0.05% Tween 20, block for 3 h with PBS + 2.5% bovine serum albumin. Wash 3x with PBS + 0.05% Tween 20.

5 Add diluted serum in triplicates, incubate overnight at 4 °C. Wash 3x with PBS + 0.05% Tween 20.

6 Add alkaline phosphatase-labeled mouse IgM- and subclass IgG-specific antibodies (working dilutions 1:250 – 1:500 in PBS + 2.5% bovine serum albumin). Incubate at 25 °C for 1 h. Wash 5x with PBS + 0.05% Tween 20.

7 Add 100 µl per well of alkaline phosphatase substrate solution. Read optical density of each well at 405 nm at 10 min and 20 min after substrate addition.

Timing

For ELISA, 2 days

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Acknowledgements

Supported by the National Institutes of Health (ES04151 and ES06376 to M.K.), the American Cancer Society (M.K.) and the Leukemia and Lymphoma Society of America (T.E.; SCOR grant 7332-06).

Kinase MEKK1 is required for CD40-dependent activation of the

kinases Jnk and p38, germinal center formation, B cell proliferation and antibody production

by Gallagher, E. et al.

Nature Immunology (22 January, 2007)