

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

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## Method Article

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# 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

**1** 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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