In vitro class switching staining for flow cytometry

CURRENT STATUS: POSTED

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DOI:
10.1038/nprot.2007.36

SUBJECT AREAS
Biological techniques

KEYWORDS
cytometry

Abstract

This protocol was used in our Nature Immunology paper.

Reagents

Frosted microscope slides (Fisher Scientific, Pittsburgh, PA)

RPMI-1640 medium (Cellgro)

ACK Lysing Buffer (Cambrex)

Cell strainers, 70 µm nylon (Becton Dickinson)

CD43 (Ly-48) magnetic beads and separation columns (Miltenyi)

B cell complete medium:

RPMI-1640 medium (Cellgro) plus 2 mM glutamine (Gibco) and 100 U/ml of penicillin g/ml and 100 µg/ml of of streptomycin (Gibco), 10% fetal calf serum (heat inactivated; Sigma), and 5×10-5 M 2-mercaptoethanol (Sigma).

24-well flat bottom culture plates (Nunc)

Anti-CD40 (clone 3/23; Pharmingen)

Recombinant mouse IL-4 (Peprotech)

Fc blocker (anti-CD16/CD32; Pharmingen)

Biotinylated anti-IgG1, anti-IgG2a-2b (both Pharmingen)

Streptavidin conjugate (Pharmingen)

FACS buffer: PBS + 0.5% fetal calf serum + 5mM EDTA

Procedure

Perform the following steps at 4 ^{o}C unless indicated otherwise.

1 Harvest splenocytes by grinding of spleens in RPMI-1640 medium between two frosted microscope slides. Spin cells at 1000 RPM for 5 min. Remove erythrocytes by adding one equivalent volume of ACK Lysing Buffer to the remaining pellet. Gently resuspend, swirl tube for 1 min. Fill tube with 10 ml of RPMI-1640, centrifuge at 1000 RPM for 5 min. Resuspend in 10 ml of RPMI-1640. Filter through 70 µm nylon cell strainer. Centrifuge at 1000 RPM for 5 min. Resuspend 1 × 107 cells in 500 µl of FACS buffer. Obtain B cells by CD43 negative purification using anti-CD43 magnetic beads.

2 Dilute CD43- B cells to a concentration of 5×105 cells/ml in B cell complete medium. Use 24-well

plates. Incubate 5×105 B cells in presence of 10 µg/ml of anti-CD40 (clone 3/23), plus 10 ng/ml of recombinant mouse IL-4 for IgG1 analysis (6). Incubate for 4 d in a humidified 37 ^{o}C, 5% CO2 incubator.

3 Wash cells with FACS buffer. Preincubate cells on ice for 10 min in presence of Fc blocker (anti-CD16/CD32). We usually use 0.5 µl of Fc blocker/ 5×105 cells in a total volume of 100 µl. CRITICAL STEP: Good Fc blockade.

4 Stain cells with biotinylated anti-IgG1, anti-IgG2a-2b (we used the antibodies at a dilution of 1:200 in a total volume of 100 µl). Incubate on ice for 20 min. Wash 2x with FACS buffer.

5 Stain cells with streptavidin conjugate of your choosing. Wash 2x with FACS buffer.

6 Analyze by flow cytometry.

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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)