

Materials List for:

# Competitive Homing Assays to Study Gut-tropic T Cell Migration

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URL: <https://www.jove.com/video/2619>

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## Materials

<p><b>Animals:</b> C57BL/6 mice are commonly used for T cell isolation. In addition, CD45.1 and Thy1.1 congenic strains are available through Jackson Laboratories (Bar Harbor, ME).</p>
<p><b>Culture media:</b> IMDM (Iscove's Modified Dulbecco's Medium + L-Glutamine + HEPES) plus 10% <b>heat-inactivated FBS</b> (Fetal Bovine Serum, low endotoxin, Gibco®, Invitrogen, Carlsbad, CA) supplemented with 100 U/ml penicillin, 100 mg/ml streptomycin (HyClone Antibiotics, Waltham, MA), 0.5 mg/ml fungizone/amphotericin B (Gibco), and 50 mM β-mercaptoethanol.</p>
<p><b>ACK Red Blood Cell Lysis buffer</b> (RBC, 10 mM KHCO<sub>3</sub>, 150 mM NH<sub>4</sub>Cl, 0.1 mM EDTA, pH 8.0), adjust to pH 7.2-7.4 and store at room temperature).</p>
<p><b>PBS</b> (Phosphate Buffered Saline, Hyclone, Waltham, MA).</p>
<p><b>Flow cytometry (FACS) media</b> (PBS or IMDM + 2% FBS + 5 mM EDTA). When staining using Selectin-Fc chimeras, media with 2 mM Ca<sup>++</sup> should be used in all steps (including FACS acquisition). IMDM is recommended in this case.</p>
<p><b>T cell labeling and adoptive transfer:</b> <b>CFSE</b> (carboxyfluorescein diacetate, succinimidyl ester), <b>CMTMR</b> ((5-(and-6)-((4-chloromethyl)benzoyl)amino) tetramethylrhodamine) from Molecular Probes®, Invitrogen, Carlsbad, CA). 1000 x stocks should be made in DMSO (5 mM <b>CFSE</b>, 20 mM <b>CMTMR</b>) and stored at -20°C.</p>
<p><b>Polyclonal T cell activation:</b> 24-well or 96-well plates (tissue-culture treated, polystyrene, flat-bottom with lid, BD Falcon, Franklin Lakes, NJ) are incubated for 2 hours at 37°C with 50 ml PBS, respectively, containing anti-CD3 plus anti-CD28 antibodies (10 mg/mL each). Then, culture plates are washed twice with PBS and used immediately for T cell culture. Alternatively, Dynabeads coated with anti-CD3/anti-CD28 (Dynal, Invitrogen, Carlsbad, CA) can be used for T cell activation instead of plate-bound antibodies.</p>
<p><b>Flow cytometry (FACS) staining:</b> Polyclonal activation: <b>CD3</b> (145C11), <b>CD28</b> (37.51). Lineage mAb: <b>CD4</b> (L3T4), <b>CD8a</b> (Ly-2), <b>Thy1.2</b> (CD90.2/53-1.2), <b>CD45.2</b> (104). Gut-homing receptors: purified <b>CCR9</b> (CD199/eBioCW-1.2, eBioscience, San Diego, CA), <b>a4b7</b> (LPAM-1/DATK3), <b>isotype control</b> (IgG2a, k). Skin-homing receptors: <b>P-selectin-Fc</b> (Purified Mouse P-Selectin - IgG Fusion Protein, BD Pharmingen, San Jose, CA), <b>E-selectin-Fc</b> (Recombinant Mouse E-Selectin/Fc Chimera, R&amp;D Systems, Minneapolis, MN) plus corresponding secondary reagent <b>goat F(ab')<sub>2</sub> anti-human IgG R-PE</b> (Invitrogen, Carlsbad, CA).</p>
<p><b>All-trans retinoic acid</b> (Sigma, St. Louis, MO) is resuspended in absolute ethanol or DMSO using a yellow bulb or an indirect source of light during the preparation. Store aliquots in glass vials at -80°C and protected from light at all times. Synthetic RAR-agonists: Am80 (Wako Chemicals, Richmond, VA).</p>