

Materials List for:

Rat Mesentery Angiogenesis Assay

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Materials

<p>Buffers and reagents</p> <p>0.5 M Tris-HCl (pH 7.8) stock solution Tris (hydroxymethyl) aminomethan (60.57 g) is dissolved in ~900 ml <i>aqua dest</i> and ~45 ml of 25% HCl (concentrated) is added to achieve pH 7.8 followed by dilution to 1 liter with <i>aqua dest</i>.</p> <p>Tris-buffer saline (TBS; pH 7.8) To 200 ml of the stock solution is added 18 g NaCl dissolved in ~1800 ml <i>aqua dest</i> with adjustment of pH to 7.8 using the stock solution, followed by dilution to 2 liter with <i>aqua dest</i>.</p> <p>TBS (pH 7.4) To 200 ml of the stock solution is added 18 g NaCl dissolved in dissolved in ~1800 ml <i>aqua dest</i> with adjustment of pH to 7.4 using the stock solution, followed by dilution to 2 liter with <i>aqua dest</i>.</p> <p>Trypsin solution (0.05% trypsin, 0.1% CaCl₂) Contains 0.1 g trypsin and 0.2 g CaCl₂ in 200 ml TBS (pH 7.8).</p> <p>Sucrose solution 2.5% Contains 5 g sucrose in 200 ml TBS (pH 7.8).</p> <p>Stock solution of 1% Sodium azide in TBS (pH 7.4) Contains 1 g Na-azide in 100 ml with TBS (pH 7.4).</p> <p>Diluting buffer: 0.1% BSA, 0.01% Na-azide (Used to dilute antibodies in the Vectastain ABC kit). Contains 0.1 g bovine serum albumin (BSA) and 1 ml of 1% Na-azide, diluted to 100 ml with TBS (pH 7.4).</p> <p>ABC kit Vectastain PK 4002, ABC peroxidase mouse IgG Normal horse serum; blocking serum; <i>yellow solution</i>: 3 drops/10 ml dilution buffer. Biotinylated antibody; anti-mouse IgG; <i>blue solution</i>: 1 drop/10 ml dilution buffer. ABC-reagent: <i>orange solution</i>, 2 drops plus <i>brown solution</i> 2 drops/10 ml TBS (pH 7.4).</p> <p>Mix these solutions 20 minutes before use!</p> <p>Mouse (monoclonal) anti-rat endothelium(Biosource International, Camarillo, CA, U.S.A.)</p> <p>This antibody is directed against all vascular endothelial cells in the rat, with the exception of those in the brain.</p> <p>Vectastain peroxidase Avidin Biotin Complex (ABC) kit The kit contains primary antibody, biotinylated secondary antibody, avidin-biotinylated enzyme complex (ABC), and enzyme substrate. See www.vectorlabs.com or www.immunkemi.se for protocols.</p> <p>Bovine serum albumin(BSA) (Sigma-Aldrich, catalog # A-4503). DAB= 3,3'-Diaminobenzidine Isopac (Sigma-Aldrich, catalog # D 9015). Trypsinfrom Bovine Pancreas (Sigma-Aldrich, catalog # T 4665). Vectastain peroxidase ABC-kit, Mouse IgG (Immunkemi F & D AB, Jarfalla, Sweden)</p>
