

Materials List for:

A Technique to Simultaneously Visualize Virus-Specific CD8+ T Cells and Virus-Infected Cells *In situ*

Qingsheng Li¹, Pamela J. Skinner², Lijie Duan¹, Ashley T. Haase¹

¹Department of Microbiology, Medical School, University of Minnesota

²Department of Veterinary and Biomedical Sciences, University of Minnesota

Correspondence to: Ashley T. Haase at haase001@umn.edu

URL: <https://www.jove.com/video/1561>

DOI: [doi:10.3791/1561](https://doi.org/10.3791/1561)

Materials

<p>Solutions and Reagents:</p> <ul style="list-style-type: none"> • PBS-H Phosphate buffered saline containing heparin (100ug/ml or 18.7U/ml) to inhibit RNAses • RPMI tissue culture media containing heparin (100ug/ml or 18.7U/ml) to inhibit RNAses • 4% low melt agarose in PBS • PBS with 2% normal goat serum (or serum from species in which the fluorophore conjugated antibodies were made). • PBS with 2% normal goat serum and 0.3% triton X-100 • MHC class I tetramers conjugated to FITC • Anti-FITC antibodies, e.g. BioDesign rabbit anti-FITC • Fluorophore conjugated anti-rabbit IgG that has been highly cross adsorbed to other species IgG for use with multiple labeling, e.g. goat-anti-rabbit-Cy3 • Fluorophore conjugated anti-mouse IgG that has been highly cross adsorbed to other species IgG for use with multiple labeling • Fresh PBS buffered 4% paraformaldehyde • 0.01M Urea • Glycerol/gelatin containing 4mg/ml n-propyl gallate
<p>Special equipment</p> <ul style="list-style-type: none"> • Vibratome • Scalpel • Razor blades • Surgical scissors • Loctite Quick Set Instant Adhesive (product # 46551) • Forceps • #2 camel hair paint brushes. Can trim with razor blade to desired thickness • 24-well flat bottomed tissue culture plates e.g. Falcon catalog #353226 • Tissue chambers • Tin foil • Cardboard slide folder e.g. Fisher catalog#12-587-10 • Confocal Microscope
<p>IST Reagents Quick Reference</p>
<p>PBS-H (phosphate buffered saline with heparin)</p> <ul style="list-style-type: none"> • 450 ml 1X PBS • 50 ml 1X PBS + 10X heparin
<p>1x PBS + 10X heparin</p> <ul style="list-style-type: none"> • 500 ml 1X PBS • 500 mg heparin powder (found on dry chemical storage shelf)
<p>PBS-H/ 2% NGS (normal goat serum)</p> <ul style="list-style-type: none"> • 49 ml PBS-H in Falcon tube • 1 ml NGS (found in 1 ml aliquots in -20 freezer)

PBS-H/ 2% NGS/ 0.3% Triton X-100
<ul style="list-style-type: none"> • 49 ml PBS-H/ 0.3% Triton X-100 in Falcon tube • 1 ml NGS (found in 1 ml aliquots in -20 freezer)
PBS-H/ 0.3% Triton X-100
<ul style="list-style-type: none"> • 500 ml PBS-H • 1.5 ml Triton X-100 (this is a thick liquid found on dry chemical storage shelves)
4% LMP Agarose
<ul style="list-style-type: none"> • 2 g LMP agarose powder • 50 ml PBS • Shake until mixed, microwave until powder dissolves.
GG-NPG (glycerol gelatin with n-propyl galate
<ul style="list-style-type: none"> • Place a bottle of glycerol gelatin in 50 degree water bath to melt • Add 0.06 g of propyl galate (found on Terri's shelf), shake well • Keep in water bath, shaking occasionally until dissolved. • Aliquot into 1 ml tubes (brown tubes)
4% paraformaldehyde
<ul style="list-style-type: none"> • 4 g paraformaldehyde powder (found on dry chemical storage shelves) • Put about 10 ml PBS-H in a graduated cylinder, add pf powder • Add some water until volume reaches about 70 ml • Add one dropper of NaOH. • Stir until dissolved (usually about 20-30 minutes) • Add HCl until pH reaches between 7-8. • Bring volume to 100 ml with water.
Urea 0.01 M
<ul style="list-style-type: none"> • 0.3 g urea (found on dry chemical storage shelves) • 500 ml water