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Common Reagents, Buffers, and Supplies for Flow Cytometry

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Blood Collection for Rodents

Prior to collection of blood from rodents from cardiac puncture, you must consider whether you want to use a blood collection tubes containing anti-coagulant. For collection of serum, collection tubes without anti-coagulants must be used. If you are intending on performing a short-term functional assessment of cells, like intracellular cytokine staining (ICS) or phosphoFlow, make sure to use SODIUM HEPARIN, SODIUM CITRATE, or appropriate non-chelating anticoagulant. Use of EDTA (chelating) can prevent cellular activation but is perfectly fine to use for phenotyping assays. As blood can clot quickly, you may even want to utilize syringes filled with anticoagulant or coated capillary tubes, depending on the IACUC-approved method of blood collection. Regardless, make sure to mix blood thoroughly after introducing the anticoagulant, by gently inverting at least 10 times in the appropriate tubes. The following are some of the options for blood collection.

- Micro sample tube, serum CAT, 1.3 ml, screw cap
- Micro sample tube Lithium heparin, 1.3 ml, screw cap
- Micro sample tube EDTA, 1.3 ml, screw cap
- 1.3ml Sodium Citrate Tube-Screw Cap
- S-Monovette(R) from Sarstedt

RBC Lysis

To obtain enriched white blood cells, you may want to lyse red blood cells (RBCs). Post incubation with lysis buffers, YOU MUST add 10-fold volume of PBS into the mixture to reverse the osmolarity to prevent lysis of white blood cells. The most commonly used lysis buffer is ACK (Ammonium-Chloride-Potassium) lysis buffer. This buffer can be prepared using in-house chemicals:

ACK Red Blood Cell Lysis Buffer (for 1 L)

Chemical	Symbol	Quantity	Concentration	Company	Catalog #
Ammonium Chloride	NH_4Cl	8.29 g	0.15 M	Sigma	A4514
Potassium bicarbonate	$KHCO_3$	1 g	1.0 mM	Sigma	P7682
Sodium EDTA	NA_2EDTA	37.2 mg	0.1 mM	Bio-Rad	161-0729

1) Add 800 mL H_2O and adjust pH to 7.2-7.4 with 1N HCl
2) Add H_2O to 1 L
3) Filter sterilize through a 0.22 µm filter and store at room temperature

Standardized RBC lysis buffers can also be purchased for improved quality control. REMEMBER to pay close attention to the manufacturer's recommended incubation time, as mouse (shorter, 1-3 minutes typically) and human (longer, 5+ minutes) have significantly different appropriate incubation times and recommended temperatures.

- ACK Lysing Buffer
- RBC Lysis Buffer (10x)
- RBC Lysis/Fixation Solution (10x)
- PharmLyse Buffer

Staining/Wash Buffers

Making your own Staining Buffer is very easy (1x PBS without Ca/Mg, 1% FBS, 0.1% Sodium Azide). Sodium Azide is toxic and comes in powder or liquid. Purchase the liquid form for ease of use. If powder is only availability, make a dilution inside a fume hood to prevent accidental inhalation. Bovine Serum Albumin (BSA) can be substituted for fetal bovine serum (FBS). The typical shelf life of a homemade buffer is ~ 1 month when stored refrigerated at 4°C. However, if you want standardized staining buffers, we recommend either of the following.

- Cell Staining Buffer
- BD Pharmingen Staining Buffer
- 10% Sodium Azide, 100mL, Sterile

Brilliant Dye Stain Buffers

If your panel contains multiple Brilliant Violet (BV) dyes, you can consider purchasing this buffer. Brilliant Violet polymer dyes can have undesired interactions with each other. Fluorescent dye interactions may cause staining artifacts which may affect data interpretation. This buffer is added during the staining process when using multiple BV dyes together in the same panel to avoid these interactions.

- Brilliant Stain Buffer
- Brilliant Stain Buffer Plus (lower volume of buffer required)
- True-Stain™ Multi-Fluor Buffer

Fixable Viability Dyes

Fixable viability dyes bind to free amine groups (R-NH2) of cellular proteins. These dyes are cell impermeant and do not pass through the cell membrane of live cells. However, late apoptotic and therefore label cells with compromised cell membrane integrity.

This process must be performed at the beginning of the protocol, especially if you plan on performing intracellular stains for cytokines or nuclear proteins (i.e. Foxp3). In fact, it is recommended that you stain your samples with these dyes prior to Fc Block or antibody surface staining, as antibodies have free amines.

Prepare these dyes with PBS because flow staining buffer contains FBS, which will sop up the dye due to the presence of proteins. Further, you should also titrate these to the extent that your live cells do not stain positive for the dye and you get good signal to noise (dead to live). You can begin with 1:100 dilution in PBS as a starting point. For Cytek Aurora panel design, we often recommend Zombie NIR or Live Dead/Blue.

Live/Dead	**Zombie**	**Ghost**
Fixable Aqua	Aqua	Violet - 450
Fixable Blue	Green	Violet - 510
Fixable Green	NIR	Violet - 540
Fixable Red	Red	Red - 710
Fixable Violet	UV	Red - 780

Fixable Yellow	Violet	UV - 450
	Yellow	Blue - 516

Non-Fixable Viability Dyes

If cells are not going to be fixed or cannot be fixed for experimental reasons but still would like to differentiate between live and dead cells, we recommend using one of the following dyes that will fit within your panel. Check out the [INFO] for wavelength information. These are DNA-intercalating dyes and will enter dead cells or cells with compromised membrane and bind to DNA. These will not enter live cells or cells with intact cell membranes.

- DAPI [INFO]
- 7-Aminoactinomycin D (7-ADD) [INFO]
- DRAQ7 [INFO]
- Propidium Iodide (PI) [INFO]
- Helix NP Dyes
- SYTOX Blue Dead Cell Stain
- SYTOX Green Dead Stain
- SYTOX Orange Dead Stain
- SYTOX Red Dead Stain

Compensation Beads

Compensation beads are used to prepare single color controls. Beads are useful for when samples contain too few cells to also prep single-stained controls or for rare populations.

For antibodies of mouse, rat, and hamster origin, and are immunoglobulin light chain-independent:

- UltraComp eBeads™ Compensation Beads
- SpectraComp Beads (Slingshot Bio)

For fixable viability dyes (amine reactive):

- ArC™ Amine Reactive Compensation Bead Kit

For fixable viability dyes (amine reactive) AND non-fixable nucleic acid dyes:

- VIACOMP (Slingshot Bio)

Cell Strainers

It is crucial that the samples are in single cell suspension. Filter your cell suspension through one of the strainers. If you are fixing samples, filter post-fixation.
Strainers that fit onto your pipette tips

- FlowmiR Cell Strainers (40 um or 70 um)
- Alternative vendor for Flowmi Cell Strainers
- Easystrainer
- CellTrics filters

Strainer that fits onto 5 mL round bottom 12 x75mm tubes

- FalconR 5 mL Round Bottom test tube with Cell Strainer Snap Cap

Strainers for 50 mL conical or 6 well plates

- CorningTM Sterile cell Strainers

Viability Dyes (Early Apoptosis)

Combining viability dye with Annexin V will detect phosphatidylserine (PS) externalization with early apoptotic cells typically positive for Annexin V but negative for the viability dye, while late apoptotic/necrotic cells will be positive for both markers.

Annexin V is a calcium-binding protein that binds to PS, which is a phospholipid on the outer leaflet of cell membranes. Cells that have externalized PS are indicative of those undergoing early phases of apoptosis. ApoTracker from BioLegend binds to the same PS as Annexin V and serves the same function.

- ApoTracker Tetra
- ApoTracker Green
- Any Annexin V conjugated reagent

Fc Block

Fc Block reagents are used to prevent fluorochrome-conjugated antibodies from binding to Fc receptors on cells (particularly Fc expressing antigen presenting cells), which would lead to false positive signal. Stain your sample with Fc Block just after fixable viability dye staining and before surface antibody staining.

- TruStain FcX (anti-mouse CD16/32) Antibody
- TruStain FcX PLUS (anti-mouse CD16/32) Antibody
- Human TruStain FcX
- TruStain FcX PLUS (Human IgG4 anti-mouse CD16/32) Recombinant Antibody

Monocyte Blocker

Monocytes and macrophages can bind to a selection of tandem dyes and produce false-positive signals, not from the antibody binding to the cells, but due to the dye itself binding to the cells. Therefore, you can use Monocyte Blocker, which is added at the same time as fluorochrome conjugated antibodies to prevent reported binding of tandem dyes. This buffer is formulated to block non-specific binding of PE/Dazzle™ 594, PE/Cyanine5, PE/Cyanine7, APC/Cyanine7, APC/Fire™ 750, and PE/Fire™ 744 commonly seen on monocytes and macrophages. The blocker is most effective when doing surface staining on live cells prior to the fixation step in a staining protocol.

- True-Stain Monocyte Blocker
- BD MonoBlock
- The Invitrogen™ CellBlox™ Blocking Buffer
  (used to prevent nonspecific binding of Invitrogen™NovaFluor™ antibodies)

Intracellular Cytokine Staining Buffer Kits

Are you looking to perform intracellular cytokine staining? Cells must be stimulated *ex vivo* with a mitogen (antigen-specific or polyclonal) and incubated with an inhibitor (Brefeldin A or Monensin, or both) to prevent cytokine secretion, allowing levels to build within cells. Usually there is a pre-

incubation period with the activator (1h), followed by a longer period with the inhibitor (4-6h). Note that inhibitors such as Brefeldin can be toxic, so should be titrated appropriately and not used in an excess of time.

- Brefeldin A
- Monensin
- CytoFix/CytoPerm
- CytoFix/CytoPerm (GolgiPlug = Brefeldin A)
- CytoFix/CytoPerm (GolgiStop = Monensin)
- Cyto-Fast Fix/Perm Buffer Set

Transcription Factor Staining

More stringent permeabilization is critical for staining nuclear transcription factors like FoxP3, RORgt, etc. These are the kits we often recommend for transcription factor detection. Remember that the antibodies that are used for intracellular staining need to be added to the cells in the permeabilization buffer and cells should be washed in the permeabilization buffer after staining to optimally remove unbound antibody.

- eBioscience Foxp3/Transcription Factor Staining Buffer Set
- True-Nuclear Transcription Factor Buffer Set

Phospho Antibody Staining Buffer Kit

Cellular activation, specifically protein phosphorylation status, can be measured by flow cytometry. Cells are typically stimulated *in vitro* or *ex vivo* for a short period of time, fixed and permeabilized and incubated with fluorescently labeled antibodies that specifically bind to phosphorylated signaling proteins. These buffers can be relatively stringent, so test that the fluorescent signal from antibodies is maintained after using these buffers. Some vendors provide information on whether antibody clones/fluors are stable after fixation with these buffers.

- True-Phos Perm Buffer
- BD Phosflow

Fixative

Paraformaldehyde (1-4%) is appropriate for fixing most samples (generally 30-minute incubation time). Purchasing an EM-grade supply and diluting with PBS, prevents you from having to prepare paraformaldehyde from powder in the lab. We typically recommend purchasing Catalog # 15712, as 10-10mL ampules are provided and can be prepared and diluted appropriately daily. Fix your cells in 1% PFA/PBS at the end of your protocol and prior to bringing your samples to the cytometer.

- Paraformaldehyde Aqueous Solution EM Grade

Cell Permeant Nucleic Acid Dyes (Cell Identification and Cell Cycle)

Cell permeant nucleic acid dyes are helpful for determining which events have nuclei in live (not fixed) complex sample with lots of debris or background. DNA-specific dyes like Vybrant DyeCycle dyes can be used to evaluate cell cycle in live cells. In fixed samples, these dyes can be used to evaluate cell cycle.

- Hoescht 33258, pentahydrate (bis-benzimide)
- DRAQ5 [Other options: Biolegend, Abcam, BD Biosciences]
- SYTO 9 Green Fluorescent Nucleic Acid Stain
- Vybrant DyeCycle Orange Stain
- Vybrant DyeCycle Ruby Stain

- Vybrant DyeCycle Violet Stain

Multiplex Kits (for Luminex)

Invitrogen **ProcartaPlex** and **Milliplex kits**assays are antibody and magnetic bead-based multiplex immunoassays that leverage Luminex xMAP technology to enable the simultaneous detection and quantitation of up to 80 protein targets in a single well with as little as 2 µL of plasma, serum, cell culture supernatants, or other bodily fluids. The assays cover more than 600 cytokines, chemokines, growth factors, and other protein targets from a range of species.