THE UNIVERSITY OF TEXAS MD Anderson Cancer Center
Making Cancer History®

NEW USER TRAINING

ANTIBODY STAINING GUIDELINES

PROCEDURE

1.0 Cell Preparation

1.1 Cells should be prepared into single cells suspensions. Cell preparation varies by sample type, and each experiment must be optimized. General guidelines can be seen below:

A. **Tissue, Tumor, and Organ:** Place tissue in media with serum and homogenize by crushing, slicing, and mincing tissue with a scalpel, passing through a blunt end needle, and filtering through 100 µm mesh. Centrifuge at 350 g for 5-10 minutes. Digest the tissue with enzymes such as collagenase, trypsin-EDTA, and DNase at 37°. Centrifuge at 350 g for 5-10 minutes. Filter cells through 70 µm filters. If necessary (i.e. spleen, lymph node), lyse RBCs with 1X lysis buffer for 2-10 minutes at room temperature. Wash with 1X PBS, centrifuge at 400-450 g for 5 minutes, and decant or aspirate supernatant.

Note: The volume of digestion enzyme and lysis buffer will depend on sample mass.

B. **Bone Marrow:** Disperse clumps, then filter cells through 70 µm filter. Lyse RBCs with 1X lysis buffer for 2-10 minutes at room temperature. Wash with 1X PBS, centrifuge at 400-450 g for 5 minutes, and decant or aspirate supernatant.

C. **Blood:** Lyse RBCs with 1X lysis buffer for 10 minutes at room temperature. Wash with 1X PBS, centrifuge at 400-450 g for 5 minutes, and decant or aspirate supernatant.

D. **Cell Line and Ascites:** Add trypsin to adherent cells for up to 10 minutes. Wash all cells with 1X PBS, centrifuge at 400-450 g for 5 minutes, and decant or aspirate supernatant.

1.2 Obtain a cell count so that antibody staining can be adjusted according to cell number.

2.0 Cell Surface Staining

2.1 Always titrate antibodies for new lots and new panels.

2.2 If you are using an antibody that tends to bind non-specifically, use an Fc block. Follow manufacturer recommendations for volume, time, and temperature of Fc block.

2.3 Add all surface antibodies to cells, leave one tube of cells unstained to use as a negative control. Include FMO (Fluorescence Minus One) tubes for any low expressing markers or rare cell types. If you have multiple samples to stain, consider making an antibody cocktail. Use Table 1 to help track how much antibody to add to each sample.

Note: Tandem dyes in cells or antibody cocktails are only stable for 1-2 days.

2.4 Stain cells for 30 minutes on ice, protected from light.

2.5 Wash with 1X PBS, centrifuge at 400-450 g for 5 minutes at 4°C, and decant or aspirate supernatant. Resuspend in 100-500 µl of FACS buffer, media, PBS, or 1% formaldehyde. Store at 4°C and protected from light until you are ready to acquire data.

Note: Formaldehyde will fix cells.

Table 1: Antibody Staining Notes

Antibody	Vol. per Tube	Vol. for Cocktail

3.0 Intracellular Staining

3.1 Some surface antigens will lose binding ability after fixation. If you are staining both surface and intracellular markers you must stain surface markers **before** fixing.

3.2 Always titrate antibodies for new lots and new panels.

3.3 Centrifuge cells at 400-450 g for 5 minutes at 4°C, decant or aspirate supernatant, add 1 ml of a fixative such as 1% formaldehyde or ice cold ethanol, and incubate for 15 minutes at room temperature.

Note: Fixation and permeabilization procedures will vary depending on assay. Follow manufacturer recommendations.

3.4 Centrifuge cells at 400-450 g for 5 minutes at 4°C, decant or aspirate supernatant, add 1 ml of a permeabilizing agent such as saponnin, Triton-X, or NP-40, and incubate for 15 minutes at room temperature.

3.5 Centrifuge cells at 400-450 g for 5 minutes at 4°C, decant or aspirate supernatant, and add all intracellular antibodies to cells. If you have multiple samples to stain, consider making an antibody cocktail. Use Table 1 to help track how much antibody to add to each sample.

Note: If antibody must be diluted it should be done in permeabilization buffer.

3.6 Stain cells for 30 minutes on ice, protected from light.

3.7 Wash with 1X PBS, centrifuge at 400-450 g for 5 minutes at 4°C, and decant or aspirate supernatant. Resuspend in 100-500 µl of 1% formaldehyde. Store at 4°C and protected from light until you are ready to acquire data.

4.0 Single Color Controls

4.1 Add compensation beads to a tube for each antibody in the panel. Follow manufacturer recommendations.

4.2 For a live/dead control add either two drops of positive green cap ArC beads to a tube, or a mixture of live and heat-treated cells. To heat treat cells, incubate cells at 65°C for 30 minutes.

4.3 Add each antibody to its labeled tube. You may need to add less antibody to the beads than was added to cells. Determine the amount of antibody needed to obtain the same MFI as positive cell populations.

4.4 Stain beads and/or cells for 30 minutes at room temperature, protected from light. Wash with 1X PBS, centrifuge at 400-450 g for 5 minutes at 4°C, and decant or aspirate supernatant.

4.5 Add 2 drops of white cap negative ArC beads to the live/dead control tube.

4.6 Resuspend in 250 µl of 1% formaldehyde. Store at 4°C and protected from light until you are ready to acquire data.

Note: Prepared compensation beads may be stable for several days depending on the fluorochrome.

RELATED PROCEDURES

This handout is related to ACSF SOP EX002. It is a handout only.