

# Protocol: Immuno-fluorescent Staining for Flow Cytometry

## MATERIAL

- Round bottom test tubes (12x75mm)
- Purified Primary or Conjugated Primary antibodies
- If needed, conjugated secondary antibody
- Anti-CD16/CD32 for Fc-blocking for mouse tissues

## BUFFERS

- Cell Staining Buffer: 1X PBS, 5%FBS, 0.1% azide
- Sheath fluid for flow cytometer

## INSTRUMENTS

- Pipettes and pipettors
- Ice bucket, refrigerator
- Centrifuge
- Flow cytometer

## METHODS

### I. Immunofluorescent staining of cell surface antigens for FACS Analysis

1. Harvest and prepare cells or tissues (spleen, lymph node, BM, thymus) as a single cell suspension\*. Add cell staining buffer and centrifuge 4-5 min, 350 x g at 4°C and discard supernatant. If using splenocytes, perform an RBC lysis step and stop the reaction with cell staining buffer. (\*Except for cultured cells, cells from tissues must be mechanically separated or by enzymatic digestion to produce single cell suspension.)
2. Wash cells with cell staining buffer. Count viable cells and resuspend at 1 x 10<sup>6</sup> cells/ml. Transfer 50µl to test tubes. Controls include: negative (no stain added), isotype control (with similarly labeled, non-specific primary antibody), and positive controls.
3. Blocking Fc Receptors is useful to eliminate non-specific immunofluorescent staining. For mouse cells, pre-incubate 0.5-1µg of anti-mouse CD16/CD32 per one million cells for 5-10 min on ice prior to staining blocks non-specific staining.
4. Add the optimally diluted conjugated, purified, or biotinylated primary antibody. Incubate on ice for 15 to 30 min in the dark.
5. Wash 3X with 2ml cell staining buffer, spin for 5 min (300-400 x g) at 4°C.
6. Resuspend cells in 500µl cell staining buffer and analyze cells.
7. If using purified or biotinylated antibodies, add the appropriate second step reagent in 100µl of staining buffer. Incubate at 4°C in the dark for 15-30 min. Repeat steps 5 and 6.

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8. Add viability dye to identify dead cells. Cells must be > 90% viable.

## II. Intracellular staining for FACS analysis

1. Prepare cells to be analyzed. Stain cell surface antigens if required. Proceed with cell surface antigen staining protocol as listed above.
2. Add 100µl of fixative (1X PBS, 0.1% formaldehyde), incubate at 4°C for 15 to 30 min. Spin and decant supernatant carefully.
3. Add 100µl of permeabilizing buffer (1X PBS with 0.5% saponin) buffer and add the fluochrome-conjugated primary antibody. Incubate at 4°C for 30 min in the dark. Treat permeabilized cells gently, spin and decant.
4. Wash 2X with cell staining buffer. Spin cells and decant supernatant each time.
5. Resuspend cells in 500µl cell staining buffer and analyze on flow cytometer.

Note: The fixation and permeabilization treatment will significantly change the FSC/SSC distribution compared to live cells.

# ABOUT



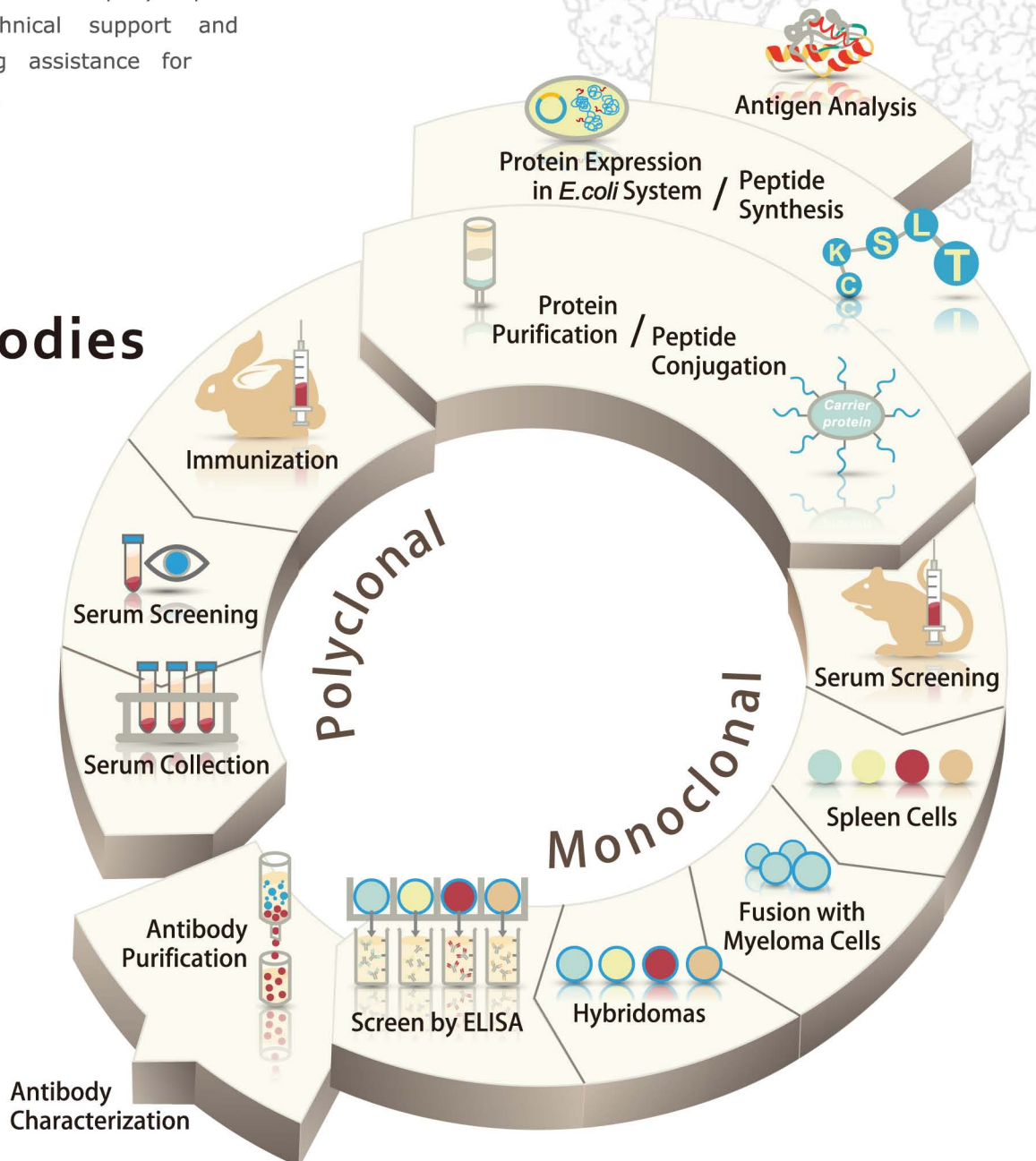
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