



DISCOVERY

L I F E S C I E N C E S

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Flow Cytometry Analysis of Dissociated Tumor Cells (DTCs)

Reagents:

- **FACS Buffer** (PBS + 2% FBS, or similar)
- **Fc Blocking Solution** (BioLegend Human TruStain FcX, or similar)
- **Fluorescently-Tagged Antibodies**
- **Viability Dye** (Propidium Iodide, DAPI, 7AAD, or similar)
- **Discovery Life Sciences Dissociated Tumor Cells (DTCs)**

Equipment:

- **Flow Cytometer**
- **Centrifuge**
- **Micropipettes**
- **Pipettors**

Procedure:

1. Thaw DTC samples as described in Thawing Viable Cell Products.
2. Resuspend cells at $0.5-1 \times 10^6$ cells/mL in FACS Buffer
3. For each stain, aliquot 0.5-1 mL cell suspension into 5 mL round bottom tubes.
 - a. For rare cell populations, staining greater than 1×10^6 cells may be required.
4. Centrifuge at 300xg for 5 minutes at room temperature
5. Decant the supernatant and gently wick away any residual buffer
6. Resuspend cells in Fc Blocking Solution according to the manufacturer's protocol.
 - a. For BioLegend Human TruStain FcX, prepare a stock solution of 25 μ l FACS Buffer + 2.5 μ l Human TruStain FcX per sample to be stained.
 - b. Resuspend cells in 25 μ l Human TruStain FcX Solution.
 - c. Incubate 15 minutes at room temperature.

Flow Cytometry Analysis of Dissociated Tumor Cells (DTCs) - Continued

7. Stain cells with fluorescently-tagged antibodies.
 - a. If multiple samples are to be stained, generate a master mix with 25 μ l FACS Buffer + the antibodies of interest per sample.
 - i. All antibodies should be titered to ensure optimal staining concentration.
 - b. Add 25 μ l staining master mix to each sample. Mix gently.
 - c. Incubate 30-45 minutes at 4°C
8. Add 1 mL FACS Buffer to each tube.
9. Centrifuge at 300xg for 5 minutes at room temperature
10. Decant the supernatant and gently wick away any residual buffer.
11. Resuspend cells in 300 μ l FACS Buffer containing a viability dye at the recommended concentration.
12. Proceed to flow cytometry analysis