# **Protocol** - Flow Cytometric Analysis of Dissociated Tumor Cells

## 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)
- **Conversant Bio Dissociated Tumor Cells** (DTCs)
    - All Conversant Bio Viable Cell Products are shipped on dry ice and should be used immediately or stored in liquid nitrogen. For more information, please refer to our document Recommended Handling and Storage for Biological Specimens.

## Equipment:

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

## Protocol:

1. Thaw DTC samples as described in Thawing Viable Cell Products.
2. Resuspend cells at 0.5-1x10^6 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 1x10^6 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 µl FACS Buffer + 2.5 µl Human TruStain FcX per sample to be stained.
    b. Resuspend cells in 25 µl Human TruStain FcX Solution.
    c. Incubate 15 minutes at room temperature.
7. Stain cells with fluorescently-tagged antibodies.
    a. If multiple samples are to be stained, generate a master mix with 25 µl FACS Buffer + the antibodies of interest per sample.
        i. All antibodies should be titered to ensure optimal staining concentration.
    b. Add 25 µl 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 µl FACS Buffer containing a viability dye at the recommended concentration.
12. Proceed to flow cytometry analysis.