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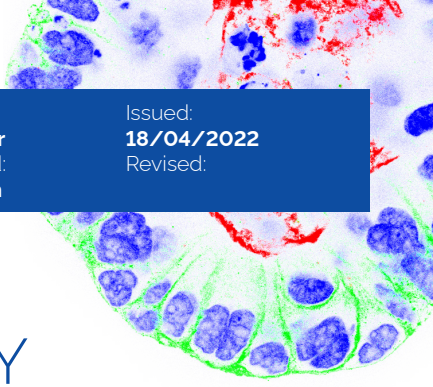

SOP –MUI-2.0	Author: **F. Mildner** Approved: **A. Amann**	Issued: **18/04/2022** Revised:

LUNG SPHEROID FLOW CYTOMETRY

Purpose

The purpose of SOP 1.0 is to describe how to analysis cell subsets using flow cytometry (FC)

Scope

SOP 1.0 is intended to cover all resources, personnel and equipment in the lung cancer organoids laboratory.

Introduction

Flow cytometry can be used to determine different cell subsets and expression profiles. This analysis allows a high throughput characterization of cells. The unit has performed routine flow cytometry and establishment of different extra- and intracellular markers.

1. Cell culture media, reagents and solutions

- Phosphate-buffered saline (PBS) (Corning, New York, USA)
- Liberase™ (Sigma Aldrich)
- Flow cytometry antibodies for specific antigens (ThermoFisherScientific, Miltenyi, BD, Biolegend)
- Foxp3 / Transcription Factor Staining Buffer Set (eBioscience™, Waltham, MA USA)
- DMEM (Corning, New York, United States)

2. Equipment

- FACS Symphony (BD Biosciences)
- FACS Tubes (5ml, BD Biosciences)
- High-speed centrifuge (Hettich Rottina 35R)
- Pipette aid, serological pipettes
- FlowJo (BD Biosciences)

3. Reagent setup

- Digestion solution: Liberase™ (Sigma Aldrich)/Stempro (without supplements) solution 1:10
- FACS staining buffer: 500 ml PBS containing 1% FBS and 2mM EDTA
- Stop solution: DMEM containing 50 % of FBS

4. Procedure

4.1 Human lung dissociation and generating 3D spheroids

- Single cell suspension from lung cancer spheroids: Transfer 20-40 (for larger FACS panels a greater number of spheroids are needed) spheroids in a 5ml FACS tube and incubate the tissue 15min at 37°C on a shaker with 1ml Liberase™ (Sigma Aldrich) in PBS 1:10.
- Gently pipette cells up and down to loosen the cells
- Add 2 ml of stop solution
- Centrifuge at 300 RPM for 5 minutes
- Decant the supernatant carefully
- Wash cells with 2ml PBS containing 1% FBS and 2mM EDTA
- Centrifuge at 300 RPM for 5 minutes
- Decant the supernatant carefully

- Repeat wash steps two times with 2ml of FACS staining buffer
- Decant the supernatant carefully
- Bring to a final volume to 100 µl with FACS staining buffer
- Add pre-titrated volume of selected antibodies to the suspension (use standard dilutions as recommended by reagent providers
- Add 2µl of 100% animal (e.g mouse) serum to inhibit unspecific binding of antibodies
- Incubate for 30 min at 4°C in the dark
- Wash cells two times with 2ml of FACS staining buffer
- Immediately measure cells with the FACS Symphony
- Analyze cells with FlowJo (See *Figure 1*)

4.2 Intracellular staining

- For intracellular staining after extracellular staining wash cells with 2ml PBS without EDTA or FBS
- Add 1ml Permeabilization/Fixation Buffer (FoxP3 Staining buffer)
- Incubate for 30 min at 4°C in the dark
- Wash two times with 2ml permeabilization buffer (FoxP3 Staining buffer)
- Bring cell into suspension in 100 µl with permeabilization buffer
- Add pre-titrated volume of intracellular FACS antibodies and 2µl of 100% mouse serum; use dilutions as recommended by reagent providers.
- Incubate for 30 min at 4°C in the dark
- Wash two times with 2ml Permeabilization Buffer (FoxP3 Staining buffer)
- Immediately measure cells with the FACS Symphony
- Analyze cells with FlowJo

5. Figures

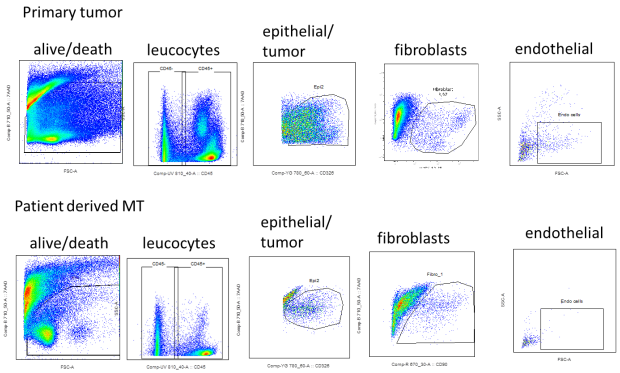

Figure 1. Representative image of gating strategy in FlowJo to discriminate between tumor cells and stromal compartments