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Staining of Dissociated Lung Cells for Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE sequencing)

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We use this protocol and it's working

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Abstract

This article describes step by step protocol for antibody-staining frozen lung cells for CITE sequencing.

Thawing of frozen dissociated human lung cells

1 Frozen cells were thawed in 37°C water bath and soon after the freezing medium melted cells were transferred into a 15 ml conical tube and the tubes were filled with staining buffer [1% BSA (w/v, Millipore) in DPBS (Biowhittaker)] to dilute the DMSO in freezing medium.

2 Cells were centrifuged at 800g, 4°C for 10 minutes.

3 After centrifugation supernatants were discarded and cells were resuspended in 1.0 ml staining buffer.

Fc receptor blocking and antibody staining

4 Viable cell counts were determined by trypan blue exclusion assay

5 Cells were then incubated in staining buffer containing 2% v/v normal mouse serum (Millipore Sigma) for 10 minutes over ice for Fc-receptor blocking.

6 Cells were then washed with staining buffer and incubated at a final concentration of 1×10^6 cells per 100 µl of staining cocktail containing analyte-specific antibodies (**attached table**) for 30 min at 4°C. Covid Supp CITE Seq Staining Antib… 14KB

7 After antibody-staining, cells were washed three times (800g, 4°C for 10 minutes) and resuspended in 100 µl staining buffer and passed through a 100-µm strainer (Falcon, Corning) and sent Genomic Research Center, University of Rochester Medical Center for further processing for CITE sequencing.