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## 709.1 Staining of Dissociated Lung Cells for Cellular Indexing of Transcriptomes and Epitopes by Sequencing (CITE sequencing) V.2

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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  $1 \times 10^6$  cells per 100  $\mu$ l 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  $\mu$ l staining buffer and passed through a 100- $\mu$ m strainer (Falcon, Corning) and sent Genomic Research Center, University of Rochester Medical Center for further processing for CITE sequencing.