🔒 BRIDGE- HTAPP_Depletion of CD45+ cells from ovarian cancer ascites single cell suspensions for single-cell RNA-Seq 👤

Benjamin Izar^{1,2}, Parin Shah^{1,2}, Mei-Ju Su^{1,2}, Isaac Wakiro¹, Sara Napolitano³, Jingyi Wu^{1,2}, Sébastien Vigneau¹, Asaf Rotem^{1,2}, Orit Rozenblatt-Rosen^{4,2}, Bruce Johnson^{1,2}, Aviv Regev^{4,5,6,7}

¹Dana-Farber Cancer Institute; ²Human Tumor Atlas Pilot Project;

³University of California, Santa Barbara; ⁴Broad Institute;

⁵Human Tumor Atlas Project; ⁶Massachusetts Institute of Technology;

⁷Howard Hughes Medical Institute

DOI:
[dx.doi.org/10.17504/protocol.s.io.4cqgsvw](https://doi.org/10.17504/protocol.s.io.4cqgsvw)

Protocol Citation: Benjamin Izar, Parin Shah, Mei-Ju Su, Isaac Wakiro, Sara Napolitano, Jingyi Wu, Sébastien Vigneau, Asaf Rotem, Orit Rozenblatt-Rosen, Bruce Johnson, Aviv Regev . BRIDGE- HTAPP_Depletion of CD45+ cells from ovarian cancer ascites single cell suspensions for single-cell RNA-Seq.

protocols.io
<https://dx.doi.org/10.17504/protocols.io.4cqgsvw>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Created: Jun 18, 2019

Last Modified: Jun 21, 2019

PROTOCOL integer ID:
24688

Judit Jane-Valbuena
The Broad Institute

ABSTRACT

This protocol is adapted from the CD45 MicroBeads MACS Separation protocol from Miltenyi Biotec (Cat No 130-045-801). CD45+ cells are labeled with microbeads following which, on passing through a MACS column in a magnetic field, they are retained within the column while the CD45- cells flow through and are collected for single-cell RNA-seq.

GUIDELINES

- Keep sample on ice and use cold reagents unless noted otherwise.
- Report information as indicated in the protocol.

MATERIALS

MATERIALS

- ⊗ LS Columns **Miltenyi Biotec Catalog #130-042-401**
- ⊗ PBS pH 7.4 **Thermo Fisher Scientific Catalog #10010049**
- ⊗ ULTRAPURE 0.5M EDTA pH 8.0 **Thermo Fisher Scientific Catalog #15575020**
- ⊗ ANTI-HUMAN CD45 MICROBEADS **Miltenyi Biotec Catalog #130-045-801**
- ⊗ BSA **Cell Signaling Technology Catalog #9998S**
- ⊗ MIDIMACS SEPARATOR **Miltenyi Biotec Catalog #130-042-302**
- ⊗ MACS MULTISTAND **Miltenyi Biotec Catalog #130-042-303**

SAFETY WARNINGS

- Follow general lab safety and institutional guidelines when working with sharps and human derived samples.

BEFORE START INSTRUCTIONS

Note

DISCLAIMER

You have been granted access to an HTAPP protocol posted in the [HTAPP_LBRNCI](#) or HTAN DCC [protocols.io](#) groups.

You hereby acknowledge that as a condition of having been provided with access to these protocols prior to their publication, you are required to comply with below guidelines:

The protocols in these groups are pre-publication and are not yet ready to be shared outside HTAN. They will eventually be made open access (per HTAPP project requirements), but until then, you agree that you will not disseminate beyond your lab or publish them without written approval of the protocol developers.

If you have questions or feedback about them, please contact Ellen at egelfand@broadinstitute.org. Many thanks for your interest!

- Set centrifuge to 4°C.
- Prepare MACS buffer (PBS with 0.5% BSA, 2 mM EDTA), degas, and keep on ice or store at 4°C for up to a few weeks.
- Label 15 mL conical tube as “CD45 negative fraction”.

Quality Control

- 1 Mix 5 µL of single-cell suspension with 5 µL Trypan blue and load on hemocytometer.
- 2 Count and record the number of viable single-cells, dead single-cells, cell doublets, and whether debris are present. Take picture if possible.

	Qualit y Contr ol
Numb er of Viable Single Cells Count ed	

Number of Dead Single Cells Counted	
Number of Cell Clumps or Doubl ets Counted	
Concentration of Viable Single Cells (cells/ μ L)	
Concentration of Dead Single Cells (cells/ μ L)	
Concentration of Cell Clumps or Doubl ets (doubl ets/ μ L)	
Volume of Single Cell Suspension (μ L)	
Total Number of Viable Single Cells	
Proportion of Single Cells that are Viable (%)	

Proportion of Cell Clumps or Doublets (%)	
Description of debris (if any)	

Note

Insert Picture:

Magnetic Labeling

- 3 Centrifuge at 580 g for 5 minutes in 4°C pre-cooled centrifuge.
00:05:00 Centrifugation
4 °C Wet Ice
- 4 Remove supernatant as completely as possible without disrupting the pellet.
4 °C Wet Ice
- 5 If cell number is lower than 10 million, resuspend cell pellet in 80 µL MACS buffer. If cell number is higher than 10 million cells, resuspend cell pellet in 80 µL MACS buffer for every 10 million cells.
4 °C Wet Ice
- 6 Add 20 µL CD45 microbeads per 80 µL MACS buffer.
4 °C Wet Ice
- 7 Incubate on ice or in 4°C refrigerator for 15 minutes.
00:15:00 Labeling
4 °C Wet Ice

- 8 During the incubation, prime an LS column: attach column to a MidiMACS separator and rinse the column with 3 mL MACS buffer.
- 9 Following incubation, resuspend cell/bead suspension with 900 μ L cold MACS buffer per 100 μ L suspension.
4 °C Wet Ice
- 10 Spin suspension at 500 g for 4 minutes in 4°C pre-cooled centrifuge.
00:04:00 Centrifugation
4 °C Centrifuge
- 11 Remove supernatant as completely as possible without disrupting cell pellet.
4 °C Wet Ice
- 12 Resuspend cell pellet in 500 μ L cold MACS buffer.
4 °C Wet Ice

Magnetic Separation

- 13 Transfer suspension to the primed LS column on the MidiMACS separator and collect the effluent (CD45 negative fraction) in 15 mL conical tube.
Room temperature