HTAPP_Depletion of CD45+ cells from single-cell suspension for single-cell RNA-seq

Isaac Wakiro1,2, Sara Napolitano1,2, Sébastien Vigneau1,2, Asaf Rotem1,2, Orit Rozenblatt-Rosen3,2, Bruce Johnson1,2, Aviv Regev3,4,5,6

1Dana-Farber Cancer Institute; 2Human Tumor Atlas Pilot Project; 3Broad Institute; 4Human Tumor Atlas Project; 5Massachusetts Institute of Technology; 6Howard Hughes Medical Institute

ABSTRACT

In some tumor specimens the proportion of malignant cells is relatively low and the that of immune cells high. In these cases, we considered strategies to deplete CD45+ immune cells as a way to both enrich for epithelial cells without using specific epithelial cells markers, and to maintain the stromal cells.

The protocol described here is used for the depletion of CD45+ expressing immune cells from human tumor-derived single-cell suspensions. It is adapted from the CD45 MicroBeads MACS Separation protocol from Miltenyi Biotec.

For the Human Tumor Atlas Pilot Project (HTAPP), CD45 depletion was used to increase the recovery of malignant epithelial cells from lung non-small cell adenocarcinoma samples in which the proportion of these types of cells was low.

Description of this protocol and guidance for testing and selecting methods for processing different tumor and sample types can be found in Slyper et al.

DOI

dx.doi.org/10.17504/protocols.io.bjxjkpkn

PROTOCOL CITATION

Isaac Wakiro, Sara Napolitano, Sébastien Vigneau, Asaf Rotem, Orit Rozenblatt-Rosen, Bruce Johnson, Aviv Regev 2020. HTAPP_Depletion of CD45+ cells from single-cell suspension for single-cell RNA-seq. protocols.io

https://dx.doi.org/10.17504/protocols.io.bjxjkpkn

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
Aug 18, 2020

LAST MODIFIED
Aug 19, 2020

PROTOCOL INTEGER ID
40651

GUIDELINES

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

MATERIALS

- **UltraPure™ BSA (50 mg/mL)** Thermo Fisher
  - Scientific Catalog # AM2616
- PBS pH 7.4 Thermo Fisher
  - Scientific Catalog #10010049
- **BSA** Cell Signaling
  - Technology Catalog #9998S
- **Trypan Blue Solution 0.4% Sterile-filtered** Sigma
  - Aldrich Catalog # T8154
- Flex-Tube® 1.5 mL PCR clean colorless Eppendorf
  - Catalog # 30389212
- Tips RT-LTS-A-1000µL-/F- 768/8 Rainin
  - Catalog # 30389212
- **Centrifuge 5430 R refrigerated with Rotor FA-45-30-11 incl. rotor lid keypad 120 V/50 – 60 Hz** (US) Eppendorf
  - Catalog # 022620601
- Tips RT-LTS-A-10µL-/F/L- 960/10 Rainin
  - Catalog # 30389226
- Tips RT-LTS-A-200µL-/F/L- 960/10 Rainin
  - Catalog # 30389240
- Falcon® 15 mL High Clarity PP Centrifuge Tube Conical Bottom with Dome Seal Screw Cap Sterile 50/Rack
  - Corning Catalog # 352097
- **NanoEnTek Inc. Disposable Hemocytometer** Westnet
  - Catalog # C-CHIP
- MiniMACS Separator Miltenyi
  - Biotec Catalog # 130-042-102
- MACS MultiStand Miltenyi
  - Biotec Catalog # 130-042-303
- MS Columns Miltenyi
  - Biotec Catalog # 130-042-201
- **CD45 MicroBeads human** Miltenyi
  - Biotec Catalog # 130-045-801
- Trypan Blue solution Sigma
  - Catalog # T8154
- Stericup Quick Release-GP Sterile Vacuum Filtration System Millipore
  - Sigma Catalog # S2GPU02RE
- **Sorvall Legend RT Refrigerated Tabletop Centrifuge** Thermo Scientific
  - Catalog # 130-042-303

SAFETY WARNINGS

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

BEFORE STARTING

- Set centrifuges to 4°C.
- Label four 15 mL conical tubes as "Supernatant", "Wash", "CD45-" and "CD45- Supernatant", and two 1.5 mL Eppendorf tubes as "CD45-". Optionally, if you plan to also collect the CD45+ fraction for further analysis, label two 15 mL conical tubes as "CD45+" and "CD45+ Supernatant", and one 1.5 mL Eppendorf tube as "CD45+". Keep tubes on ice. Supernatants are collected to enable, if needed, the recovery by centrifugation of cells that have failed to pellet or were accidentally aspirated (e.g., if the final yield is too low).
- Prepare MACS buffer: PBS with 0.5% BSA and 2 mM EDTA (using Cell Signaling Technology BSA #9998S).

---

**Citation:** Isaac Wakiro, Sara Napolitano, SAAë Bastien Vigneau, Asaf Rotem, Orit Rozenblatt-Rosen, Bruce Johnson, Aviv Regev (08/19/2020). HTAPP_Depletion of CD45+ cells from single-cell suspension for single-cell RNA-seq. [https://doi.org/10.17504/protocols.io.bjxjkpkn](https://doi.org/10.17504/protocols.io.bjxjkpkn)

This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Sterile filter, degas, and keep on ice. This solution can be prepared in advance and stored at 4°C for several weeks.

Prepare PBS with 0.4% BSA (using Thermo Fisher Scientific UltraPure BSA #AM2616) and keep on ice. This solution can be prepared in advance and stored at 4°C for several weeks.

Initial 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, then calculate additional quality control metrics below. Take picture if possible.

<table>
<thead>
<tr>
<th>Initial Cell Suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Viable Single Cells Counted</td>
</tr>
<tr>
<td>Number of Dead Single Cells Counted</td>
</tr>
<tr>
<td>Number of Cell Clumps or Doublets Counted</td>
</tr>
<tr>
<td>Concentration of Viable Single Cells (cells/µL)</td>
</tr>
<tr>
<td>Concentration of Dead Single Cells (cells/µL)</td>
</tr>
<tr>
<td>Concentration of Cell Clumps or Doublets (doublets/µL)</td>
</tr>
<tr>
<td>Volume of Single Cell Suspension (µL)</td>
</tr>
<tr>
<td>Total Number of Viable Single Cells</td>
</tr>
<tr>
<td>Proportion of Single Cells that are Viable (%)</td>
</tr>
<tr>
<td>Proportion of Cell Clumps or Doublets (%)</td>
</tr>
<tr>
<td>Description of debris (if any)</td>
</tr>
</tbody>
</table>

Insert Picture:

Magnetic Labeling

3. Centrifuge cell suspension in a 1.5 mL Eppendorf tube at 500 g for 4 minutes in 4°C pre-cooled centrifuge.

4. Carefully transfer supernatant to the 15 mL “Supernatant” tube kept on ice without disturbing the pellet.

5. If the cell pellet contains less than 10^7 cells, resuspend in 80 µL MACS buffer. If the cell pellet contains more than 10^7 cells, resuspend in 80 µL MACS buffer per 10^7 cells.

6. Add 20 µL CD45 microbeads per 80 µL MACS buffer.

Volume of Microbeads Used (µL):

Volume of MACS Buffer Used (µL):

Citation: Isaac Wakiro, Sara Napolitano, Sébastien Vigneau, Asaf Rotem, Ort Rozenblatt-Rosen, Bruce Johnson, Aviv Regev (08/19/2020). HTAPP_Depletion of CD45+ cells from single-cell suspension for single-cell RNA-seq. https://doi.org/10.17504/protocols.io.bjxjkpkn

This is an open access protocol distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
7. Incubate in 4°C refrigerator for 15 minutes.

8. During the incubation, insert an MS column into the MiniMACS separator set on the MACS MultiStand. Prime the column with 500 µL MACS buffer, collecting the effluent in the 15 mL "Wash" tube.

9. Following incubation (Step 7), add 1 mL cold MACS buffer per 100 µL suspension.

10. Centrifuge suspension at 500 g for 4 minutes in 4°C pre-cooled centrifuge.

11. Carefully transfer supernatant to the 15 mL "Supernatant" tube kept on ice without disturbing the pellet.

12. If the cell pellet contains less than $10^8$ cells, resuspend in 500 µL MACS buffer. If the cell pellet contains more than $10^8$ cells, resuspend in 500 µL MACS buffer per $10^8$ cells.

13. Position the 15 mL "CD45-" tube under the MS column, discarding the "Wash" tube.

14. Transfer cell suspension (up to $2x10^8$ cells) to the primed MS column on the MiniMACS separator and collect the flow-through in the 15 mL "CD45-" tube.

15. Wash the MS column by adding 500 µL MACS buffer and collecting the flow-through in the 15 mL "CD45-" tube. Repeat wash two more times.

16. Transfer the "CD45-" tube on ice.

[Optional] Collection of the CD45+ fraction

17. Remove the MS column from the MiniMACS separator and position it on top of the 15 mL "CD45+" tube.

Citation: Isaac Wakiro, Sara Napolitano, Sébastien Vigneau, Asaf Rotem, Orit Rozenblatt-Rosen, Bruce Johnson, Aviv Regev (08/19/2020). HTAPP_Depletion of CD45+ cells from single-cell suspension for single-cell RNA-seq. https://dx.doi.org/10.17504/protocols.io.bjxjkpkn

This is an open access protocol distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Add 1 mL of MACS Buffer to the column and immediately elute the CD45+ cells by firmly pushing the plunger into the column.

Transfer the "CD45+" tube on ice.

Add 1 mL of MACS Buffer to the column and immediately elute the CD45+ cells by firmly pushing the plunger into the column.

Transfer the "CD45+" tube on ice.

Transfer the CD45- fraction to two 1.5 mL "CD45-" Eppendorf tubes on ice. (If a CD45+ fraction has been collected, transfer it to one 1.5 mL "CD45+" Eppendorf tube on ice.)

Centrifuge cell suspensions at 500 g for 4 minutes in 4°C pre-cooled centrifuge.

Tip: If the number of cells is expected to be small (e.g., less than 50,000 cells), higher recovery may be obtained by centrifugation for 8 sec at 4°C using short spin setting, with centrifugal force ramping up to but not exceeding 11,000 g (do not spin for a longer duration or at a higher centrifugal force, as this would result in cell death).

Carefully transfer supernatant from the "CD45-" tubes to the 15 mL "CD45- Supernatant" tube kept on ice without disturbing the pellet. (If a CD45+ fraction has been collected, transfer the supernatant from the "CD45+" tube to the 15 mL "CD45+ Supernatant" tube kept on ice, without disturbing the pellet.)

Resuspend and combine both CD45- cell pellets in a total volume of 50 µL cold PBS with 0.4% BSA. (If a CD45+ fraction has been collected, resuspend the CD45+ pellet in 50 µL cold PBS with 0.4% BSA.)

Final Quality Control

Mix 5 µL of single-cell suspension with 5 µL Trypan blue and load on hemocytometer.

Count and report the number of viable single cells, dead single cells, cell doublets or clumps, and whether debris are present, then calculate additional quality control metrics below. Take picture if possible.

<table>
<thead>
<tr>
<th>CD45- Fraction</th>
<th>CD45+ Fraction (if collected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Viable Single Cells Counted</td>
<td></td>
</tr>
<tr>
<td>Number of Dead Single Cells Counted</td>
<td></td>
</tr>
<tr>
<td>Number of Cell Clumps or Doublets Counted</td>
<td></td>
</tr>
<tr>
<td>Concentration of Viable Single Cells (cells/µL)</td>
<td></td>
</tr>
<tr>
<td>Concentration of Dead Single Cells (cells/µL)</td>
<td></td>
</tr>
<tr>
<td>Concentration of Cell Clumps or Doublets (doublets/µL)</td>
<td></td>
</tr>
<tr>
<td>Volume of Single Cell Suspension (µL)</td>
<td></td>
</tr>
<tr>
<td>Total Number of Viable Single Cells</td>
<td></td>
</tr>
<tr>
<td>Proportion of Single Cells that are Viable (%)</td>
<td></td>
</tr>
<tr>
<td>Proportion of Cell Clumps or Doublets (%)</td>
<td></td>
</tr>
<tr>
<td>Description of debris (if any)</td>
<td></td>
</tr>
</tbody>
</table>

Insert Picture for CD45- fraction:

Insert Picture for CD45+ fraction (if collected):

Citation: Isaac Wakiro, Sara Napolitano, Sébastien Vigneau, Asaf Rotem, Orit Rozenblatt-Rosen, Bruce Johnson, Aviv Regev (08/19/2020). HTAPP_Depletion of CD45+ cells from single-cell suspension for single-cell RNA-seq. https://dx.doi.org/10.17504/protocols.io.bjxjkpkn

This is an open access protocol distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
If necessary, adjust the concentration of the CD45- fraction before proceeding to loading on 10x Genomics Single-Cell RNA-seq system, following 10x Genomics recommendations. Tip: 8,000-10,000 live cells are typically loaded per channel. Optimal cell recovery is achieved for concentrations between 800 and 1,200 cells/µL but deviations from that range are acceptable (see 10x Technical Note on this topic). Furthermore, it is recommended that viability be higher than 60% and the proportion of cell clumps lower than 5%.

Report the information listed below about loading on 10x Genomics Single-Cell RNA-seq system, including the number and concentration of cells per channel.

Time of Loading:

Person Loading:

Single-Cell RNA-seq Kit Used:

Concentration of Viable Cells Loaded (cells/µL):

Number of Cells Loaded per Channel:

Number of Channels Loaded: