Version 2

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HTAPP_Depletion of CD45+ cells from ovarian cancer ascites single cell suspensions for single-cell RNA-Seq V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bhbij2ke

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ABSTRACT

Many ovarian cancer ascites samples have a high proportion of CD45-expressing cells (>90%) (CD45+) and a low proportion (<10%) of malignant cells. In such cases, depletion of CD45+ immune cells can be used to increase the proportion of malignant cells, as well as other non-immune cell types, without relying on the expression of epithelial or tumor-specific markers.

For the Human Tumor Atlas Pilot Project (HTAPP) this protocol was used to deplete CD45+ immune cells from single-cell suspensions obtained from ovarian cancer ascites, and is included as a step in the "HTAPP_Processing human ovarian cancer ascites to a single-cell suspension for single-cell RNA-seq" protocol, also available in protocols.io. It is adapted from the CD45 MicroBeads MACS Separation protocol from Miltenyi Biotec (Cat No 130-045-801).

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.

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PROTOCOL CITATION

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PARENT PROTOCOLS

In steps of

HTAPP_Processing human ovarian cancer ascites to a single-cell suspension 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

NAME	CATALOG #	VENDOR
LS Columns	130-042-401	Miltenyi Biotec
PBS pH 7.4	10010049	Thermo Fisher Scientific
ULTRAPURE 0.5M EDTA pH 8.0	15575020	Thermo Fisher Scientific
ANTI-HUMAN CD45 MICROBEADS	130-045-801	Miltenyi Biotec
BSA	9998S	Cell Signaling Technology
MIDIMACS SEPARATOR	130-042-302	Miltenyi Biotec
MACS MULTISTAND	130-042-303	Miltenyi Biotec
Falcon® 15 mL High Clarity PP Centrifuge Tube Conical Bottom with Dome Seal Screw Cap Sterile 50/Rack 500/Case	352097	Corning

SAFETY WARNINGS

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

BEFORE STARTING

- Set centrifuge to 4˚C.
- Prepare MACS buffer (PBS with 0.5% BSA and 2 mM EDTA), sterile filter, degas, and keep on ice. This solution can be prepared in advance and stored at 4˚C for several weeks.
- Label a 15 mL conical tube as “Wash”.
- Label a 15 mL conical tube as “CD45 negative fraction”.

Quality Control

1 Mix 5 µL of red blood cell-free ascites single-cell suspension with 5 µL Trypan blue and load on hemocytometer. The ascites cell suspension can be obtained following the "HTAPP_Processing human ovarian cancer ascites to a single-cell suspension for single-cell RNA-seq" protocol.

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

	Quality Control
Number of Viable Single Cells Counted	
Number of Dead Single Cells Counted	
Number of Cell Aggregates or Doublets Counted	
Concentration of Viable Single Cells (cells/µL)	
Concentration of Dead Single Cells (cells/µL)	
Concentration of Cell Aggregates or Doublets (doublets/µL)	
Volume of Single Cell Suspension (µL)	
Total Number of Viable Single Cells	
Proportion of Single Cells that are Viable (%)	
Proportion of Cell Aggregages or Doublets (%)	
Description of debris (if any)	

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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 cell pellet.
4 °C Wet Ice

5 If cell number is lower than 10 million, resuspend the cell pellet in 80 µL MACS buffer. If cell number is higher than 10 million cells, resuspend the 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: insert the column into a MidiMACS separator and let 3 mL of MACS buffer flow through the column, collecting it in the 15 mL "Wash" tube. Once the column is primed, discard the 15 mL "Wash" tube and position the 15 mL "CD45 negative fraction" tube under the column.

9 Following incubation (Step 7), resuspend the labeled cell suspension with 900 µL cold MACS buffer per 100 µL of suspension.
4 °C Wet Ice

10 Spin the 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 the labeled cell pellet.
4 °C Wet Ice

12 Resuspend the pellet (up to 100 million cells) in 500 µL cold MACS buffer.
4 °C Wet Ice

Magnetic Separation

13 Transfer the labeled cell suspension to the primed LS column on the MidiMACS separator and collect flow-through in

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the 15 mL "CD45 negative fraction" tube. This CD45 depleted cell suspension can be used in the "HTAPP_Processing human ovarian cancer ascites to a single-cell suspension for single-cell RNA-seq" protocol.

Tip: Cell recovery may be increased by washing the column up to three times with 3 mL of MACS buffer.

Room temperature

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