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Staining of single-cell suspensions for scMEP mass cytometry analysis

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

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ABSTRACT

Staining of single-cell suspensions for scMEP analysis by mass cytometry (CyTOF). This protocol includes 1) surface staining for 30min at RT, 2) PFA-fixation for 10min at RT, 3) MeOH-based permeabilization for 10min on ice 4) intracellular staining for 1h at RT and 5) DNA intercalation.

EXTERNAL LINK

https://www.nature.com/articles/s41587-020-0651-8

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MATERIALS TEXT

MATERIALS

Cell-ID™ Intercalator-Rh—500 µM **Fluidigm Catalog # 201103A** Step 16

EQ™ Four Element Calibration Beads **Fluidigm Catalog # 201078** Step 21

Obtain single cell suspension

1 Prepare single cell suspensions in FACS tubes as described in the following protocol:

Preparation of single-cell suspensions for scMEP mass cytometry analysis
by Felix Hartmann,
Stanford University
PREVIEW RUN

1.1 Prepare single-cell suspension with your established method of choice.

- Be aware: cell aggregates clog the small tubing of the CyTOF and prevent acquisition.
- Use of digestive enzymes might be necessary but can lead to epitope loss.
- Cell numbers depend on the experimental question but preparing 1-3 million cells per sample is recommended.

1.2 1h

Prepare stocks for small molecules:

- IdU (Recommended stock **500 Milimolar (mM)** in DMSO)
- BrU (Recommended stock **100 Milimolar (mM)** in PBS)
- Puromycin (Recommended stock **1 mg/ml** in ddH2O)

Solutions can be gently heated in a **50 °C** water bath to completely dissolve solute if necessary. Once dissolved, prepare small aliquots and store at **-20 °C** until needed.

Reagents:
IdU **Sigma Aldrich Catalog #I7125**

BrU **Sigma Aldrich Catalog #850187**

Puromycin **Sigma Aldrich Catalog #P8833**

More details:

Citation: Felix J Hartmann (03/19/2021). Staining of single-cell suspensions for scMEP mass cytometry analysis. https://dx.doi.org/10.17504/protocols.io.bntnmeme

Kimmey SC, Borges L, Baskar R, Bendall SC (2019). Parallel analysis of tri-molecular biosynthesis with cell identity and function in single cells.. Nature communications.
https://doi.org/10.1038/s41467-019-09128-7

1.3 Prepare mastermix of small molecules in cell-type appropriate medium (can be supplemented with serum or other compounds). 15m

Compound	Final concentration	Stock concentration	Volume per sample
IdU	100 uM	500 mM	0.2 uL
BrU	2 mM	100 mM	20 uL
Puromycin	5 ug/mL	1 mg/mL	5 uL
Medium (cell-type specific)			1 mL

1.4 Resuspend cells in **1 mL** of mastermix, and incubate at **37 °C** for **00:30:00** . 30m

1.5 Transfer cell suspension to FACS tubes or similar vessel and fill with cell-type appropriate medium (not containing IdU/BrU/puromycin) and centrifuge at **300 x g, Room temperature , 00:05:00** . 5m

1.6 Aspirate supernatant and resuspend cells in **1 mL** PBS. 1m

- Do not resuspend in serum containing solutions as proteins would suck-up all the live-dead reagent.

1.7 Pre-dilute Cisplatin-198Pt to **25 Micromolar (µM)** in PBS (Stock **100 Milimolar (mM)**). 5m

- First time: aliquot Cisplatin stock solution into **5 µl** aliquots and store at **-20 °C** until needed.
- Aliquots can be thawed and refrozen multiple times.
- Discard pre-diluted Cisplatin after use.

Reagents:
Cisplatin-198Pt **Fluidigm Catalog # 201198**

1.8 Add **1 µl** pre-diluted Cisplatin-198Pt to cells in PBS, vortex and incubate for **00:05:00** at **Room temperature** . 5m

- Cisplatin will enter membrane-compromised cells (i.e. dead cells) and unspecifically bind DNA as well as amine groups.

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1.9 Add **3 mL or whatever your vessel allows** of Cell Staining Medium (CSM: PBS + 0.5% BSA + 0.02% sodium azide) and centrifuge **300 x g, Room temperature , 00:05:00** . 5m

1.10 ⚠ 2m

Aspirate supernatant and loosen cell pellet by flicking tube or vortexing.

- It is important to dissovle aggregates before proceeding to prevent cell cross-linking and eventual problems during acquisition.

1.11 Dilute 16% PFA to 1.6% in PBS. 2m

- PFA from Electron Microscopy Sciences has been shown to work well.
- Opened 16% PFA can be stored airtight and light-protected (e.g. in black falcon tube) for up to two weeks.
- Diluted 1.6% PFA should be discarded after use.

Reagents:
16% paraformaldehyde **Fisher Scientific Catalog #50-980-487**

1.12 Add **1 mL** of freshly diluted 1.6% PFA to loosened cells, mix well and incubate for **00:10:00** at **Room temperature** to fix cells. 10m

1.13 Add **3 mL or whatever your vessel allows** of Cell Staining Medium (CSM) and centrifuge **600 x g, 4°C, 00:05:00** . 5m

1.14 Aspirate supernatant and resuspend cells in **1 mL** of CSM + 10% DMSO. 5m

1.15 Transfer to cryotube and freeze at **-80 °C** . 5m

- Fixed cells can be stored like this for multiple months before staining and acquisition.

Barcoding (optional)

2 Perform palladium-based barcoding as described in the following protocol:

(still needs to be written)

Surface staining 30m

3 Prepare surface antibody mastermix.

Note: Antibodies were titrated for 3 million cells, if more cells are to be used, scale antibody amounts accordingly (i.e. use 2x antibodies for up to 6 million cells).

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Combine all surface antibodies and supplement volume to a total of **50 µl** with Cell Staining Medium (CSM: PBS + 0.5% BSA + 0.02% sodium azide). If antibody volume succeeds **50 µl** , no CSM addition is needed.

4 Add antibody mastermix to cells and mix carefully by pipetting up and down, followed by a gentle vortex. Incubate for 30m
00:30:00 at **Room temperature** .

5 Add **2 mL** of CSM, mix carefully and centrifuge
600 x g, Room temperature , 00:05:00 , Reduce to 300g when working with live cells .

6 Aspirate supernatant and dissolve pellet by flicking tube or vortexing.

Fixation 10m

7 Dilute 16% PFA to 1.6% in PBS.

- PFA from Electron Microscopy Sciences has been shown to work well.
- Opened 16% PFA can be stored airtight and light-protected (e.g. in black falcon tube) for up to two weeks.
- Diluted 1.6% PFA should be discarded after use.

Reagents:
16% Paraformaldehyde (PFA) **Fisher Scientific Catalog #50-980-487**

8 Add **1 mL** of freshly diluted 1.6% PFA to loosened cells, mix well and incubate for **00:10:00** at 10m
Room temperature to fix antibodies onto cells. Also complete this section when working with pre-fixed cells.

9 Add **3 mL or whatever your vessel allows** of Cell Staining Medium (CSM) and centrifuge
600 x g, 4°C, 00:05:00 . Aspirate supernatant and loosen cell pellet by flicking and vortexing.

Permeabilization 10m

10 Note: MeOH based permeabilization has been found to work well with intracellular metabolic antibodies although most are suspected to work with saponin-based approaches as well. 10m

While vortexing cells, drop-wise add **1 mL** of pre-cooled (**4 °C**) MeOH and immmediately transfer cells
On ice to incubate for **00:10:00** .

11 Following incubation, add **3 mL or whatever your vessel holds** of CSM and centrifuge
600 x g, 4°C, 00:05:00 . Aspirate supernatant and loosen cell pellet.

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12 Repeat washing by adding **3 mL or whatever your vessel holds** of CSM and centrifuge **600 x g, Room temperature , 00:05:00** . Aspirate supernatant and loosen cell pellet.

Intracellular staining 1h 30m

13 Prepare intracellular antibody mastermix.

Note: Antibodies were titrated for 3 million cells, if more cells are to be used, scale antibody amounts accordingly (i.e. use 2x antibodies for up to 6 million cells).

Combine all intracellular antibodies and supplement volume to a total of **50 µl** with CSM. If antibody volume succeeds **50 µl** , no CSM addition is needed.

14 Add antibody mastermix to cells and mix carefully by pipetting up and down, followed by a gentle vortex. Incubate for 1h 30m **01:00:00** at **Room temperature** . Flick tube carefully after approximately **00:30:00** to ensure proper mixing of antibodies and cells.

15 Add **2 mL** of CSM, mix carefully and centrifuge **600 x g, Room temperature , 00:05:00** . Aspirate supernatant and loosen pellet.

DNA intercalation 20m

16 Prepare intercalation solution: 1.6% PFA in PBS + **0.5 Micromolar (µM)** of Intercalator-Rh.

- Note: Iridium-based DNA intercalators can be used alternatively, however they do tend to spill into adjacent platinum channels which are often used here for live/dead discrimination as well as for antibody channels. Rhodium DNA intercalation is compatible with palladium-based barcoding.
- When using for the first time, aliquot Rh-intercalator and store at **-20 °C** until use
- Rh-intercalator can be thawed and re-frozen multiple times

Cell-ID™ Intercalator-Rh—500 µM **Fluidigm Catalog # 201103A**

17 Add **1 mL** of intercalation solution for each 10 million cells and incubate either **00:20:00** at **Room temperature** or **Overnight** at **4 °C** . 20m

Acquisition

18 First CSM wash step: Add **3 mL or whatever the vessel holds** of **CSM** to the cells and centrifuge **600 x g, Room temperature , 00:05:00** . Aspirate supernatant and loosen cell pellet.

19 Second ddH2O wash step: Add **3 mL or whatever the vessel holds** of **ddH2O** to the cells and centrifuge **600 x g, Room temperature , 00:05:00** . Aspirate supernatant and loosen cell pellet.

20 Third ddH20 wash step: Add **3 mL or whatever the vessel holds** of **ddH2O** to the cells and centrifuge

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600 x g, Room temperature , 00:05:00 . Aspirate supernatant and loosen cell pellet.

21 Dilute EQ beads 1:10 in ddH2O and add **1 mL** of diluted beads per 1 million cells.

EQ™ Four Element Calibration Beads **Fluidigm Catalog # 201078**

22 Filter cells through cell strainer and acquire on CyTOF

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