Mar 19, 2021

Preparation of single-cell suspensions for scMEP mass cytometry analysis

Felix J Hartmann[1]

[1]Stanford University

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

Felix Hartmann
Stanford University

SUBMIT TO PLOS ONE

ABSTRACT

Preparation of single cell suspensions to be analyzed for metabolic and phenotypic features by scMEP mass cytometry analysis. This protocols spans 1) optional incubation of cells with small molecules to be able to asses biosynthesis rates of DNA, RNA and protein 2) cisplatin-based live/dead staining 3) PFA-based cell fixation and 4) cryopreservation.

EXTERNAL LINK

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

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Hartmann FJ, Mrdjen D, McCaffrey E, et al. Single-cell metabolic profiling of human cytotoxic T cells (2020). *Nature Biotechnology*. doi:10.1038/s41587-020-0651-8

DOI

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

EXTERNAL LINK

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

PROTOCOL CITATION

Felix J Hartmann 2021. Preparation of single-cell suspensions for scMEP mass cytometry analysis . **protocols.io** https://dx.doi.org/10.17504/protocols.io.bkwkkxcw

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Hartmann FJ, Mrdjen D, McCaffrey E, et al. Single-cell metabolic profiling of human cytotoxic T cells (2020). *Nature Biotechnology*. doi:10.1038/s41587-020-0651-8

KEYWORDS

scMEP, single-cell metabolism, CyTOF, mass cytometry, immunometabolism

CREATED

Sep 05, 2020

LAST MODIFIED

Mar 19, 2021

PROTOCOL INTEGER ID

41644

PARENT PROTOCOLS

In steps of

Staining of single-cell suspensions for scMEP mass cytometry analysis

MATERIALS TEXT

MATERIALS

DMSO **Contributed by users**

PBS **Contributed by users**

5-Iodo-2'-deoxyuridine (IdU) **Sigma Aldrich Catalog #I7125**

5-Bromouridine (BrU) **Sigma Aldrich Catalog #850187**

Puromycin **Sigma Aldrich Catalog #P8833**

Cisplatin-198Pt **Fluidigm Catalog # 201198** Step 7

16% Paraformaldehyde (PFA) **Fisher Scientific Catalog #50-980-487** Step 11

Phosphate buffered saline (PBS) **Contributed by users**

Obtain single-cell suspension

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.

Analysis of biosynthesis rates (this section is optional) 1h

1h

2 ⊛

Prepare stocks for small molecules:

- IdU (Recommended stock [M]**500 Milimolar (mM)** in DMSO)
- BrU (Recommended stock [M]**100 Milimolar (mM)** in PBS)
- Puromycin (Recommended stock [M]**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:

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

IdU **Sigma**
Aldrich Catalog #I7125

BrU **Sigma**
Aldrich Catalog #850187

Puromycin **Sigma**
Aldrich Catalog #P8833

More details:

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

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

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

Live / dead staining 10m

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

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.

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.

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

- Aliquots can be thawed and refrozen multiple times.
- Discard pre-diluted Cisplatin after use.

Reagents:

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

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.

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

Cell fixation 20m

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.

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 (PFA) **Fisher Scientific Catalog #50-980-487**

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

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

Freezing 10m

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

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

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

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

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