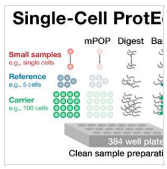

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SCoPE2

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ABSTRACT

Protocol for preparing single cells for mass-spec analysis by SCoPE2 as described by Specht et al., doi: 10.1101/665307 (2019) and Specht et al., *Genome Biology,* doi: 10.1186/s13059-021-02267-5 (2021).

For a detailed protocol paper, see
Petelski A, Emmott E, Leduc A, Huffman RG, Specht H, Perlman D, Slavov N (2021)
Multiplexed single-cell proteomics using SCoPE2
Nature Protocols (in press)

DOI

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EXTERNAL LINK

https://genomebiology.biomedcentral.com/articles/10.1186/s13059-021-02267-5

PROTOCOL CITATION

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Specht, H., Emmott, E., Petelski, A.A. et al. Single-cell proteomic and transcriptomic analysis of macrophage heterogeneity using SCoPE2. Genome Biol 22, 50 (2021). https://doi.org/10.1186/s13059-021-02267-5

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Prepare 384-well plate(s) for sorting single cells

1

Add 1uL of 25 fmol/uL Waters MassPrep (dissolved in HPLC-grade water) to each well of a 384-well thermalcycler plate (ThermoFisher AB1384).

2 Seal plate with adhesive foil (Thermo Fisher Scientific; cat. no: AB0626)

Cell sorting

3 CRITICAL: Ensure that cell sorter lines contain only PBS and have not been supplemented with any proteinaceous mixture like FBS or Serum Albumin. Once cells are in suspension, wash 3x with ice-cold PBS prior to sorting.

We have successfully isolated individual cells using CellenONE, BD FACSaria, Sony MA900, and MoFlo Astrios.

Sort single cells into the prepared plates. Spin down, and then immediately freeze at -80 C.

Bulk cells: Carrier and reference channel preparation

4 Isobaric carrier and reference channels can be prepared from sorted cells or harvested straight from cell culture. In either case, removing proteinaceous cell culture media is necessary; as stated above, washing cells in ice cold PBS or saline at least 3x prior to further steps.

We recommend both channels be close in composition as possible to the single cells. We recommend preparing at least three times as much material as you need for the single cell experiments. So if you plan to prepare 384 single cells, that would be 32 SCoPE2 sets labeled by TMTpro (each containing 12 single cells). This would require 32 x 200 cells worth of carrier and 32 x 5 cells worth of reference. Multiply this number by at least 3 to be safe.

4.1 Dilute cells to 2000 cells / ul in mass spec grade water.

4.2 Freeze for 20 min at -80 C, heat for 10 min at 90 C in a thermal cycler (or other device). Allow to cool to room temperature.

4.3 Add triethylammonium bicarbonate pH 8.5 (TEAB) to final concentration 100 mM concentration
Add bezonase to final concentration 0.2x activity units
Add trypsin to final concentration 10ng/ul

Allow to digest overnight at 37 C with shaking.

Overnight

4.4 Take 1 ul of the resulting digest and analyze by MS. Search raw file with MaxQuant and analyze with DO-MS. Ensure that miscleavage rate < 20%. See the DO-MS report tab "Carrier".

4.5 If <20%, proceed. If not, re-add the same amount of trypsin as the first step and re-digest overnight.

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4.6 Separate 5/200th = 1/40th of the sample, this will be the reference channel.

4.7 Label the carrier material with TMT126 and the reference with TMT127N. 1h

Dilute TMT tags to 1:4 the manufacturer recommended concentration in acetonitrile.

Add mass tags so that their final concentration is 30-40% v/v

Incubate at RT with shaking.

01:00:00

4.8 Take 1ul of each carrier and reference. Analyze by MS. Search raw file with MaxQuant and analyze with DO-MS. Ensure that TMT labeling efficiency > 95%. See the DO-MS report tab "Carrier".

4.9 If >95%, proceed. If not, re-do TMT labeling step.

4.10 Add hydroxylamine to final concentration 0.1% v/v 45m

Incubate at RT with shaking for **00:45:00**

4.11 Combine carrier and reference together. Dry down in SpeedVac.

Single cell: mPOP Lysis

5 Preheat a thermal cycler to 90 C, heat lid to 105 C.

Transfer plate from -80C to preheated thermal cycler quickly.

00:10:00

Single cell: Digestion and Labeling

6 Spin down plate at RT, sonicate in water bath for 5 minutes.

7 Spin down on benchtop centrifuge to collect liquid.

8 ON ICE: Make a solution of Trypsin Gold, benzonase and triethyl ammonium bicarbonate pH 8.5 (TEAB) such that in the digestion volume TG will be present at 10ng/ul and TEAB will be present at 100 mM concentration and benzonase at 0.2x concentration.

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Add 0.2uL of 20 ng/uL TG in 200 mM TEAB pH8.5, 0.4x benzonase, to each reaction well.

9 Cover. Vortex 10s. Sonicate 10s.

Spin to collect liquid.

10 Digest in thermal cycler for 3-18 hours at 37 C, lid 55 C.

03:00:00

11 Spin to cool to room temperature.

12 Remove the TMT labels from the -80 and allow to come to room temperature

Dilute 85mM TMT labels 1:4 in acetonitrile.

Add 0.5uL of the resulting solution to wells. (We have tested this concentration of TMT for up to 200 cells.)

Cover. Vortex 10s. Sonicate 10s. Spin down.

13 Incubate for 1 hour at room temp (22 C)

01:00:00

14 Add 0.2 ul of 1% hydroxylamine.

Cover, mix, spin down.

00:45:00

15 Dilute carrier channel + reference channel mix such so that it is at 200 + 5 cells / 5 ul. Collect the volume from each single cell well by adding 5ul of the carrier + reference mixture to the first well in each set then serially passing that mixture through the remaining single cell wells in that set.

16 Pass through 5 uL 50% acetonitrile to each well in a given set and add to combined sample. The combined sample should now have 5ul of the carrier+reference mixture, 5ul of 50% acetonitrile, and 12 x ~2 ul single cell material (if using TMT16, 8 x ~2 ul if using TMT11)

17 Deposit into an MS glass insert.

18 Speedvac to evaporate your sample down to appropriate volume, we inject 1 uL out of 1.2 uL

19 Place the insert in a labled MS vial and securely cap the vial with a pierceable cap.

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Vortex your sample, then spin down.

20 Submit to QE. Method and gradient described by here: https://doi.org/10.1186/s13059-021-02267-5

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