DeMuxlet Cell Preparation Protocol

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Demuxlet Cell Preparation Protocol

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1 Works for me This protocol is published without a DOI.

Human Cell Atlas Method Development Community

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ABSTRACT

This protocol details the preparation of PBMC cells.

ATTACHMENTS

Demuxlet_Cell_Preparation_Protocol.docx

PROTOCOL CITATION

Woong-Yang Park, Jay Shin, Shyam Prabhakar 2020. Demuxlet Cell Preparation Protocol. **protocols.io** https://protocols.io/view/demuxlet-cell-preparation-protocol-bf87jrzn

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GUIDELINES

- If cell viability for any sample is <50%, avoid using that sample in the suspension mix.
- If cell viability is between 50-70%, you may try to enrich for viable cells by centrifuging at lower speed (100 g). It may improve the viability to >75%, the cells can then be mixed with the other samples.

MATERIALS TEXT

- RPMI (Glu-) (Gibco; Cat. No.: 21870076)
- Human Serum (HS) (Sigma; Cat. No.: H4522)
- FBS (Sigma; Cat.No: F2442)
- Glutamine (Invitrogen/Gibco; Cat.No.: 25030081)
- Penicillin Streptomycin (Invitrogen/Gibco; Cat. No.: 15140122)
- PBS (Ca- Mg-) (Gibco; Cat.No.: 14190144)
- BSA (Capricorn Scientific; Cat. No.: BSA-1S)
- Wide-bore blue tips (Fisher Scientific; Cat. No.: FIS #14-222-703)

- Trypan Blue 0.4% (Gibco; Cat. No.: 15250061)
- Macs SmartStrainers (30 µm),Miltenyi Biotec; Cat No.: 130-110-915)

SAFETY WARNINGS

Please refer to the Safety Data Sheets (SDS) for health and environmental hazards.

Preparation of Reagents and Media

1 Prepare appropriate amount of thawing media including RPMI + 5% HS + 1% Pen/strep + 1% glutamine and keep it for a maximum of 2 weeks at **4 °C** .

2 Prepare appropriate amount of wash media including RPMI + 10% FBS + 1% Pen/strep + 1% glutamine.

3 Prepare appropriate amount of PBS + 0.04% BSA.

Thawing Frozen PMBCs and Preparing the Suspension Mix

4 Warm up thawing media, wash media and PBS (Ca- Mg-) at **37 °C** .

5 Transfer **9 mL** of **37 °C** warm thawing media into a **15 mL falcon tube** .

6 Take the tube with PBMCs out of Liquid Nitrogen and transfer on dry ice immediately to the **37 °C** water bath and thaw for **00:01:00** to **00:02:00** .

7 After **00:01:00** to **00:02:00** , open the tube under the laminar hood and add **1 mL warm thawing media** (it's a dropwise addition) into the tube using the wide-bore blue tips.

8 Having added the warm media, transfer the whole suspension into the **15 mL falcon tube** which contains **9 mL warm thawing media** using the wide-bore blue tips.

It should be added very gently (dropwise or while you keep the tube at 45 degree).

9

Mix the solution extremely gently by pipetting (3-4 times) using the wide-bore blue tips (without any bubbling).

The cells at this stage are very fragile.

10 

Centrifuge at **300 rpm 00:05:00** .

11 Decant the supernatant.

12 Re-suspend the cells in **5 mL pre-warmed wash media** very gently.

13 Re-suspend the cell pellet very gently by pipetting (5-10 times) using the wide-bore blue tips.

14 

Centrifuge at **200 rpm 00:05:00** .

15 Decant the supernatant and re-suspend the cells in **3 mL pre-warmed PBS + 0.04% BSA.**

16 Repeat **Step 14 and 15.**

17 After the second wash, re-suspend the cells in **3 mL PBS + 0.04% BSA** .

18 Filter the cells through the 30-50 µm cell strainer (FACS tubes) to get rid of clumps or debris.

19 Count the cells and make aliquots of $1x10^6$ cells/mL (500 µL).

20 Keep the rest of the cells at **-20 °C** for DNA extraction and genotyping with Illumina Global Screening Array-24 v3.0 BeadChip (Cat. No.: 20030770) according to the manufacturer's protocol.

21 

Mix equal volumes (**100 µl**) of cell-suspension from each sample (up to 16 samples) to make a mixed suspension with a final concentration of $1x10^6$ cells/mL which will be used for 10X single cell capture.

22 Count cell number in the mixed suspension again and keep it **On ice** for further processing of single cell capturing using 10X Chromium.