Human axillary lymph node fine-needle aspirate sample processing and cyropreservation

Jacqueline HY Siu[1], cdendrou[1]

[1]University of Oxford

Jacqueline HY Siu
University of Oxford

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We use this protocol and it's working

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ABSTRACT

This protocol was used to generate a single-cell suspension from fine-needle aspirate samples of the adult human axillary lymph nodes and cyropreserved in CS10 for < 6 months. Cell yield was highly dependent on the participant; however, viability was >90% prior to freezing and between 80-90% after thawing (trypan blue dye exclusion).

GUIDELINES

- All steps should be performed at 4ºC unless stated otherwise.

SAFETY WARNINGS

Sample preparation should be carried out in a Class II microbiological safety cabinet in a designated Containment Level 2 blood handling facility. Centrifuge steps should be performed in a designated blood-handling centrifuge with aerosol-tight inner lids.

BEFORE START INSTRUCTIONS

Just prior to starting:

- Pre-cool centrifuge to 4ºC
- Pre-cool cyropreservation vials in the -20ºC
- Pre-cool cell freezing container if necessary

Reagents:

- **R10 Media** is made up with RPMI 1640 with 25 mM HEPES (Cat No: R5886, Sigma), 10% heat inactivated fetal bovine serum (HI-FBS) (Cat No: F4135, Sigma), 1% Pen/Strep (Penicillin-Streptomycin 10,000 Units/mL (Cat No: 15140-122, Gibco), and 2 mM of L-Glutamine (Cat No: 25030-024, Gibco). Sterile bottle 500 mL filter system 0.22 µM (Cat No: 430758/ 430769 Corning or Cat No: SEGPU0538/ SEGPU0545, Merck) or 0.22 µM syringe filters. Store at 4ºC when not in use for a maximum of 1 month.
- **Cell Wash Buffer** is made up with PBS with 2% human AB serum and 2 mM EDTA. Store at 4ºC when not in use.

Sample collection & transfer

1 Collect lymph node FNA samples in sterile filtered R10 media in 15 mL Falcon tubes. Transfer samples in an appropriate container and keep the tubes at 4ºC using cooled gel packs.

Sample processing

2 Upon sample arrival, top up tubes with cold RPMI + 5% human AB serum (serum has been previously heat inactivated and sterile filtered) as required to ensure even volumes. 1m

3 Centrifuge at 400xg at 4ºC for 10 min. 400 x g, 4°C, 00:10:00 10m

4 Remove the supernatant, resuspend in 5 mL of red blood cell (ACK) lysis buffer (Gibco, Cat: A10492-01). Incubate at room temperature for 5 min (check colour), up to 10 min max. Top up with cell wash buffer (PBS + 2% FBS, 2 mM EDTA). Room temperature 10m

5 Centrifuge at 400xg at 4ºC for 10 min. Wash again with cell wash buffer (PBS + 2% FBS, 2 mM EDTA). 400 x g, 4°C, 00:10:00 10m

6 Adjust final volume to 1 ml of cold RPMI + 5% AB serum. 1m

7 Take a 10µl aliquot of the cell suspension and dilute with 10µl Trypan Blue. Load this mixture onto a haemocytometer and perform a cell count. 5m

Sample cyropreservation 10m

8 Label the tubes and chill at -20ºC for ~ 10 minutes. The minimum number should be ~500,000 cells (to be frozen in 100 µl; 100 µl is the minimum freezing volume). Aliquot cells such that

there is a maximum of ~1 million cells per cryovial.

9 Add up to 10 ml of cold RPMI + 5% hAB serum to the tubes. Centrifuge at 400x*g* for 10 min at 4ºC. 10m
400 x g, 4°C, 00:10:00

10 Resuspended samples in CS10 medium at a concentration of 1 ×10^6/100 µl. 1m

11 Once fully mixed, aliquot 100 µl of the sample into the chilled cryotubes. 1m

12 Transfer the cryotubes into appropriate cell freezing container. Ensure all slots of the MrFrosty are filled, using the filler vials if necessary. 2m

13 Transfer the samples into liquid nitrogen. Ideally this transfer should be performed within 24 hr, but may be extended to a maximum of 72 hr. Overnight