

FREEZING OF CELLS

Equipment	Specification	Catalog No.	Supplier
Centrifuge	Heraeus Multifuge 35SR+		Thermo Fisher Scientific
vacuum		NA	
Freezing box	freezing rate -1°C/min; Mister Frosty	5100-0001	Nalgene
1000µL pipete	Monochanel Research Pipette, 100-1000µL, variable, manual		Eppendorf
Biosafety cabinet	HeraSafe	51025108	Thermo Fisher Scientific

Material	Specification	Catalog No.	Supplier
15mL tubes	Volume up to 15mL, sterile, PP	62554502	SARSTEDT
50mL tubes	Volume up to 50mL, sterile, PP	62547254	SARSTEDT
pipetes 5mL	Serological, pipeting volume 1-7mL	4487	COSTAR
pipetes 10mL	Serological, pipeting volume 1-13mL	4488	COSTAR
pipetes 25mL	Serological, pipeting volume 2-35mL	4250	COSTAR
Plastic Pasteur pipetes	Pipette transfert 4ml	251-15	D.DUTSHER

Reagent	Specification	Catalog No.	Supplier	Storage/validity
Human Serum Albumin (HSA), Alburex 20	20g/100mL (=20%)	Lot A50111-2960	PAA	2-8°C/36 months
DMSO	> 99,5%	P60-36720100	SIGMA	RT
RPMI 1640	+ GlutaMAX-I,500mL	61870-010	GIBCO	2-8°C/12 months

Freezing medium (95% FCS + 5% DMSO)

- Thaw FCS, de-complement it (incubate 30 min at 56°C), filter it, aliquot by 15 mL and store at -80°C.
- On the day of freezing of cells take one aliquot of frozen FCS, thaw it and add to it 0.75mL DMSO. Close the tube and invert it 3x to obtain homogeneous solution.
- Store at 2-8°C and use within 24h.

Note: All steps should be performed within one day and, if not specified differently, at RT. Cool freezing medium and freezing tubes at 4°C before use.

1. Count the cells and centrifuge them at 300g for 8 min at 4°C
2. Aspirate supernatant by using plastic Pasteur pipet
3. Add 1 ml of Freezing medium and mix at least 3x by pipeting up and down
4. Add 1ml of freezing medium for two aliquots or 1,5ml for three aliquots and calculate the number of cells per aliquot. Report it on the document.

Note:

- PBMCs obtained by Ficoll separation from 16-18mL of blood, are in average frozen in two aliquots of 0.5mL, with a concentration of approximately 1×10^7 cells/mL
 - EBV-transformed cell lines are frozen in five aliquots of 0.5mL, with a concentration of approximately 5×10^6 cells
5. Aliquot cell suspension in freezing tubes (0.5 mL 2D tubes) using a 1 ml pipet.
 6. Scan 2D tubes
 - When freezing PBMCs fill in the “**Sample follow up**” form.
 - When freezing EBV fill in the “**EBV transformation follow up**” form
 7. Put the tubes in a Mr. Frosty cryobox previously cooled down at 4°C
 8. Transfer the cryobox immediately to -80°C freezer.

9. After at least 16h of storage at -80°C transfer frozen 2D tubes from Mr. Frosty cryobox to liquid nitrogen.
10. Note down the position of each tube in liquid nitrogen tank in the document “**Sample storage layout**”.
Note: After 30 days (4 usages) change isopropanol in Mr Frosty box.
11. Prepare Mr. Frosty for future use by storing it at 4°C .

