

STANDARD OPERATING PROCEDURE

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Applicable to: ITN Project 5P	Category:

Title: Thawing Mouse Treg Cell Stocks (from the UCSF Transplantation Research Lab)

- 1) Prepare one 15 ml conical tube for each cryovial you will be thawing. Transfer 9 ml of Wash Medium to each tube. Warm these tubes in a CLEAN water bath or leave these tubes in the hood until they warm up to room temperature.
- 2) Remove vials of frozen cells from the liquid nitrogen tank, and place on dry ice while carrying to the water bath. From this point on, you should work quickly as the cells are very fragile while being thawed in DMSO.
- 3) One at a time, dip the cryovials in a 37°C water bath being sure not to submerge the cap of the vial in the water bath until 1/2 ~ 1/3 of contents are thawed. Swirl the vial around until you can see some liquid moving around a piece of ice in the vial. Wipe the outside of each tube with a Kimwipe soaked with 70% ethanol followed by a dry Kimwipe.
- 4) Transfer the vial to a hood, draw up 1 ml of warmed Wash Medium with a **serological 2-ml pipette, not P1000 pipetman**, to avoid sheer pressure and slowly add the media to the cells. Gently pipette up the cells and slowly transfer to the 15 ml conical tube containing the rest of the media. Rinse the vial once with 1 ml of media from the same conical tube and combine the cells. Cap the conical tube and gently invert the tube to mix the contents and count the cell numbers to check the viability.
- 5) Spin down the cells at 1500 rpm for 5 min at room temperature.
- 6) Use the cells for experiment

Wash medium (D2F+)

DMEM with high glucose (4.5 g/L)	500 ml (Gibco, 11960-044)
HEPES (1M)	5 ml (Invitrogen, 15630-080)
Fetal Calf Serum	10 ml
2-mercaptoethanol (1000X)	0.5 ml (Invitrogen, 21985-023)
Penicillin-Streptomycin (5,000 U/mL)	5 ml (Invitrogen, 15070-063)

REVISION HISTORY

Effective Date	Revision Version	Author	Description of Changes
Dec 2014	Version 1	Bluestone	Original Version
14-Aug-2015	Version 2	T Kupfer	Incorporated tracked changes. Corrected typos and catalog numbers.