

## Freezing Down Cells

No	Description/Details of Steps in Activity	Hazards	Possible Accident / Ill Health & Persons-at-Risk	Existing Risk Control (Mitigation)	Severity	Likelihood (Probability)	Risk Level	Additional Risk Control
1	Grow your cells in a large plate (150 mm) to a confluent monolayer.	Spillage, biological exposure to mammalian cells and endogenous viruses/blood borne pathogens,	Exposure to mammalian cells and blood borne pathogens, self-inoculation by needlestick injury	All personel handling cell lines are to have Hep B vaccination, and passage numbers are not to be maintained too high. Internal training is compulsory. wear proper PPE (gloves, lab coat, covered shoes); handle cells in the dedicated BSL2 biosafety cabinet; have disinfectant (e.g. 70 % ethanol) on hand.	1	1	2	
2	Prepare 10 ml of cryo media. This is just 95% FBS and 5% DMSO. In a 15 ml tube, add 10 ml FBS and 0.555 ml DMSO. Mix well.	Spillage and exposure to DMSO which is an irritant	Eye contact with DMSO may cause blurry vision, burning sensation, redness, tearing, and vasodilation.	Wear proper PPE (goggles, gloves, lab coat, covered shoes); handle cells in the dedicated BSL2 biosafety cabinet; dispose of any spillage using the Chemical Spill SOPs.	1	1	2	
3	You will need to wash with ~20 ml of media, and use 5 ml of trypsin (3 ml media, 2 ml trypsin). Collect the trypsin/cells into a 50 ml tube (instead of 15 ml, using 5 ml serological pipette), and wash the plate with 20 ml of serum-containing media. After you remove the media from your cells, resuspend the pellet in the 10 ml of cryo media. Aliquot 1 ml of this into each of the 10 cryotubes.	Spillage, biological exposure to mammalian cells and endogenous viruses/blood borne pathogens, injury due to improper usage of centrifuge and fingers	Exposure to mammalian cells and blood borne pathogens, self-inoculation by needlestick injury. Injury due to imbalanced centrifuge and trapping of limbs or fingers	Internal training is compulsory for centrifuge use and the centrifuge key. wear proper PPE (gloves, lab coat, covered shoes); handle cells in the biosafety cabinet; have disinfectant (e.g. 70 % ethanol) on hand. When using the centrifuge, ensure centrifuge is balanced and rotor is placed correctly, and that all tubes are capped tightly. Close centrifuge properly and ensure that there are no funny sounds when centrifuge is running. Decontaminate waste using Presept tablets (dilute to 10% solution of activaed bleach to kill cells) and let the bleach decontaminate for half an hour before discarding with plenty of water and dilution.	1	1	2	

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4	Put the tubes into the Mr. Frosty and place in the -80°C freezer. Keep in the freezer for 24 – 48 hours.	Spillage, biological exposure to mammalian cells and endogenous viruses/blood borne pathogens,	Exposure to mammalian cells and blood borne pathogens, self-inoculation by needlestick injury	All personel handling cell lines are to have Hep B vaccination, and passage numbers are not to be maintained too high. Internal training is compulsory. wear proper PPE (gloves, lab coat, covered shoes); handle cells in the dedicated BSL2 biosafety cabinet; have disinfectant (e.g. 70 % ethanol) on hand.	1	1	2	