

Thawing immortalized cells

Purpose: Immortalized cells are stored in liquid nitrogen. To begin growing them, they require gentle thawing and a rinse to get rid of DMSO in the freezing media.

Reagents:

Cell Culture Medium

1X PBS, calcium-free

Note- Be careful not to get freezing media on your gloves. DMSO in the freezing media permeates nearly any surface and will carry bacteria or viruses from your gloves into your skin.

Procedure:

1. Warm cell culture media and PBS in the water bath.
2. Prepare the supplies in the hood:
 - a. Aliquot 9ml PBS into a 15ml conical tube.
 - b. Set out a 2ml serological pipette.
 - c. Fill one well of a 6-well plate with 1ml cell media. Label the plate.
 - d. Get out a bucket of dry ice.
3. Remove the cryovial of frozen cells from liquid nitrogen onto dry ice for transport. Quickly return to the cell culture room and immediately hold the cryovial halfway-submerged in the water bath.
 - a. Be careful not to submerge the lid of the cryovial because it could introduce contaminants into the cells.
 - b. Thaw a maximum of two cryovials at one time.
4. Continue to hold the cryovial in the waterbath until the liquid inside begins to thaw. When only a small chunk of ice is left in the cryovial, bring it to the hood.
5. Slowly add 1ml PBS from the 15ml conical to the cryovial. The rest of the ice chunk should melt.
 - a. Adding the PBS slowly prevents heat shock in the cells.
6. Remove the cell suspension to the 15ml conical of PBS. Mix gently 2x.
7. Centrifuge at a low speed for ~3min until a cell pellet forms.
8. Aspirate the supernatant. Reconstitute the cell pellet in 1ml of media. Mix gently and remove to the prepared well of the 6-well plate.
9. Incubate.