Thawing frozen cell line

1. Background and purpose of the procedure

The purpose of this document is to have a standard thawing procedure for cell lines used in the laboratory.
It is vital to thaw cells correctly to maintain the viability of the culture and enable the culture to recover more quickly. Some cryoprotectants, such as DMSO, are toxic above 4˚C therefore it is essential that cultures are thawed quickly and diluted in culture medium to minimize the toxic effects.

2. Materials and equipment

- Frozen cells in cryovial
- Complete cell culture medium (containing 10-20% FBS and antibiotics depending on cell line)
- 70% ethanol

- Absorbent paper
- Biosafety cabinet
- Petri dish or tissue culture flask
- 37˚C water bath
- Pipets and tips
- Pipet gun and pipettes
- Tissue culture incubator (at 37˚C, 5% CO_2, humidified)

3. Method

This procedure needs to be performed in a sterile environment (biosafety cabinet)

a. Remove vial from liquid nitrogen and place in a water bath at 37˚C (wear lab coat, cryo-gloves, safety glasses and face shield)
   i. Submerge only the lower half of the vial
   ii. Allow to thaw until a small amount of ice remains in the vial - usually 1-2 minutes
   iii. Transfer to biosafety cabinet after wiping the vial with 70% ethanol
b. Wipe the outside of the vial with a tissue moistened (not excessively) with 70% ethanol hold tissue over vial to loosen lid
c. Slowly, dropwise, pipette cells into pre-warmed growth medium to dilute out the DMSO
d. Incubate cells overnight. Change media the next morning. Removal of DMSO is critical
e. Examine cells microscopically (phase contrast) after 24 hours and sub-culture as necessary

4. Revision history

Revision #	Date	Prepared by
1.0	**2021-07-14**	**Elie Besserer-Offroy**
Summary of modifications		
Initial version of the protocol		