

Freezing and thawing mammalian cell lines

Freezing cells

Trypsinise or collect cells as normal for subculture procedure. Collect cells in complete media and centrifuge at 1000g for 5 minutes. Resuspend cell pellet in freezing media and aliquot this into cryovials. Freeze for short term use at -80C (maximum 6 months) or store in liquid nitrogen for long term storage.

Typically, 1 15 cm plate for HeLa cells can give rise to 3-4 1ml cryovials.

Freezing media (general)

90% FBC + 10% DMSO.

Specialised media (RCC4, fragile cell types)

70% Complete media, 20% FBS, 10% DMSO.

Thawing cells

Gently defrost the cryovial in a 37C water bath. Prepare small plate with complete media that has been pre-warmed. For robust cells, place content of the cryovial directly into the new late. For fragile cells, wash defrosted cells in complete media using a falcon tube and gentle centrifugation prior to placing cells in the new plate.