Cryopreservation of Mammalian Cells

Tips:

a) The following protocol describes a general procedure for the cryopreservation of cultured cells.

b) Prepare freezing medium and store at 2° to 8°C until use. Note that the appropriate freezing medium depends on the cell line.

c) Freeze the cultured cells at a high concentration with a low passage number as possible. Make sure that the percent viability of the cultured cells is more than 90% before freezing. [Note]: The optimal freezing conditions depend on the cell line in use.

d) Store the frozen cells below –70°C; frozen cells begin to deteriorate above –50°C.

e) Always use sterile cryovials for storing frozen cells and the cryovials containing the frozen cells should be stored immersed in liquid nitrogen or in the gas phase above the liquid nitrogen.

f) Please pay attention to personal protective measures.

Protocol: Suspension cells

a) Count the number of viable cells to be cryopreserved and calculate required volume of freezing medium according to the viable cell density [Note]: Cells should be in log phase.

b) Centrifuge the cells at 500 × g for 5-10 min, discard the supernatant and collect the cell pellet.

c) Resuspend the cells with freezing medium at a concentration of 1 x 10^6 cells/mL.

d) Aliquot the cell suspension into sterile cryovials and place the vials on wet ice or in a 4°C refrigerator, and start the freezing procedure within 5 minutes.

e) Cells are frozen slowly at 1°C/min. This can be achieved with **a programmable cooler** or by placing vials in **an insulated box** placed in a –70°C to –90°C freezer for overnight.

Then transfer the cryovials to liquid nitrogen for storage.

Protocol: Adherent cells

a) Gently detach the cells from the substrate with dissociation reagents (such as trypsin). (**It can be refered to the protocol of passaging adherent cells**)

b) Resuspend the detached cells with complete growth medium. Count the number of viable cells to be cryopreserved and calculate required volume of freezing medium according to the viable cell density [Note]: Cells should be in log phase.

c) Centrifuge at 500 x g for 5-10 min to pellet cells, discard the supernatant and collect the cell pellet.

d) Resuspend cells with freezing medium at a concentration of 1 x 10^6 cells/mL.

e) Aliquot the cell suspension into sterile cryovials and place the vials on wet ice or in a 4°C refrigerator, and start the freezing procedure within 5 minutes.

f) Cells are frozen slowly at 1°C/min. This can be achieved with **a programmable cooler** or by placing vials in **an insulated box** placed in a –70°C to –90°C freezer for overnight. Then transfer the cryovials to liquid nitrogen for storage.