Public Health England

ECACC
European Collection of Cell Cultures
Operated by Public Health England

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Sucessful Culture of Your Cells

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Please read the important information that follows before handling ECACC-supplied cell lines.

Storage of Frozen Cells

Upon receipt, frozen ampoules should be transferred directly to gaseous phase liquid nitrogen without delay, unless they are to be used straight away. DO NOT use a -80°C freezer as an alternative; this will result in loss of viability.

How to Handle Frozen Cells on Receipt

Upon receiving a shipment of frozen ECACC-supplied cell lines it is important that the end user gives the shipment attention without delay. The technical advice accompanying the cell lines should be consulted before removing ampoules from the dry ice. Correct handling immediately upon receipt is critical to successfully establishing the cell line in the end user's laboratory.

At the time a cell line is ordered, end users should consider the culture conditions for the new cell line and make sure the appropriate medium will be available when the cells arrive.

Importance of Cell Counting

Perform a viable cell count when you resuscitate and harvest cell cultures. One of the most common reasons for the failure to establish cells in culture is due to using an incorrect viable cell seeding density at the time of resuscitation i.e. seeding cells too low or too high. This can be avoided by performing a viable cell count and following the recommended seeding density.

Read the cell line data entry for the specific cell line you are working with, available on the ECACC website at www.phe-culturecollections.org.uk. The seeding density is shown in the subculture routine information. When seeding cells immediately post resuscitation, use the mid to upper end of the seeding density range given.

Resuscitation of Frozen Cells

It is important to handle frozen ampoules with care; wear a laboratory coat, full protective face mask and gloves. On rare occasions ampoules may explode on warming due to expansion of trapped residual liquid nitrogen.

1. In a microbiological safety cabinet, hold a tissue soaked in 70% alcohol around the cap of the frozen ampoule. Turn the cap a quarter turn to release any residual liquid nitrogen that may be trapped. Retighten the cap. Quickly transfer the ampoule to a 37°C water bath until only one or two small ice crystals, if any, remain (1-2 minutes). It is important to thaw rapidly to minimise any damage to the cell membranes.

Note: Do not totally immerse the ampoule as this may increase the risk of contamination.

2. Wipe ampoule with a tissue soaked in 70% alcohol prior to opening.

3. Pipette the whole content of the ampoule into a sterile tube (e.g. 15ml capacity). Then slowly add 5ml pre-warmed medium that has already been supplemented with the appropriate constituents. Determine the viable cell density using trypan blue stain, a haemocytometer and an inverted microscope to count the cells or equivalent cell counting method. Transfer the appropriate volume of cell suspension to achieve the cell seeding density recommended on the cell line data entry.

For adherent cell lines: Adjust the volume of the medium, and if necessary the flask size, to achieve the cell seeding density recommended on the cell line data sheet. A pre-centrifugation step to remove cryoprotectant is not normally necessary as the first media change will remove residual cryoprotectant. If it is, then this will be specified on the data sheet. If the cells are to be used immediately (e.g. for a cell based assay), rather than subcultured, it may be advisable to perform a pre-centrifugation step to remove cryoprotectant.

For suspension cell lines***:** A pre-centrifugation step to remove cryoprotectant is recommended i.e. pellet the cells by centrifugation at 150 *x g* for 5 minutes and resuspend the cell pellet in fresh medium using the appropriate volume to achieve the correct seeding density.

1. Incubate flasks at the temperature and CO_2 level recommended on the data sheet. If a CO_2 fed incubator is used the flask should have a vented cap to allow gaseous exchange.

Hybridoma Cultures From Frozen

When recovering hybridoma cultures from frozen it is not unusual for growth initially to be slower than expected and there may be an observed decrease in viability. Establishment of an actively proliferating culture may take up to 2 weeks.

On resuscitation a centrifugation step to remove the cryoprotectant is essential. Rapidly thaw the frozen ampoule in a water bath at 37°C for 1-2 minutes. Transfer the contents to a centrifuge tube and slowly add 5-10ml of pre-warmed growth media^{+}. Remove a sample for counting. Centrifuge at 100 *x* g for 2-3 minutes to pellet cells and seed at a relatively high density of 5-7 x 10^5 cells/ml. Place culture flask flat and observe regularly until viable proliferating cells are seen.

+Often hybridoma cultures may benefit from being resuspended with media supplemented with 20% FBS in the early critical stage of culture establishment immediately post resuscitation.

Procedure for Freezing Cells

ECACC recommends freezing a stock of your cell line(s) soon after receipt (between 2 - 4 x 10^6 cells/ml) as a precaution.

The following guide is offered for the preparation and cryopreservation of cell lines.

1. Harvest the cells in the log phase of growth in the same manner used for routine subculture. For adherent cell lines, harvest as close to 80-90% confluency, as possible.

2. The standard procedure is to use 90% serum + 10% cryoprotectant for all cell lines unless otherwise specified on the data sheet. Allow 1ml for each ampoule. Most cell lines can be frozen in the appropriate culture medium supplemented with 20% serum and 10% cryoprotectant. This is usually DMSO but in certain instances glycerol is recommended. If DMSO is not suitable an alternative will be specified on the cell line data sheet.

3. Pellet cells by centrifugation e.g. 150 *x g* for 5 minutes. Resuspend the cell pellets in the appropriate freeze medium to give a final cell concentration between 2 - 4 x 10^6 cells/ml and pipette 1ml into each ampoule.

4. Freeze the cells at a cooling rate between 1-3°C/min using a programmable rate controlled freezer or suitable alternative[1]. When the temperature reaches at least -130°C, transfer the ampoule to a gas phase liquid nitrogen storage vessel.

It is advisable to test cell viability by thawing one ampoule after short term storage in gas phase nitrogen.

Ref. Cryopreservation of Animal Cells in Advances in Biotechnology Processes (1988), 7, A.R. Liss

[1] A polystyrene box, or alcohol bath (e.g. a Nalgene 5100 'Mr Frosty' box Sigma cat no. C1562) can be used in a -80^{o}C freezer for up to 24 hours prior to transfer to gaseous phase nitrogen.