

Important technical information for successful culture of your cells

Please read before handling cell cultures

Important: This document is not applicable for induced Pluripotent Stem Cells (iPSCs) a separate document is available for iPSCs on each of the iPSC product detail pages

This document provides important guidance on:

Storage of frozen cells	1
Importance of cell counting	1
Resuscitation of frozen cells	2
Hybridoma cultures from frozen	3
Handling growing cell cultures	3
Procedure for freezing your cells	4
Certificates of Analysis	4

Read the cell line specific data on the product detail page for the cell line you have received at www.phe-culturecollections.org.uk

Storage of frozen cells

Frozen cells provided in plastic vials must be stored at below -135°C on 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.

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
- 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

Advisory note: Wear personal protective equipment including laboratory coat, protective face mask and gloves when handling the frozen vials. On very rare occasions vials may explode on warming due to the expansion of trapped residual liquid nitrogen (refer to Material Safety Data Sheet at www.phe-culturecollections.org.uk/technical)

The following guidance aims to help you establish a culture successfully and minimise cell damage and contamination:

1. Transport the vial in dry ice or in a liquid nitrogen transport vessel to maintain the vial at low temperature until you are ready to resuscitate the cells
2. Quickly transfer the vial to a 37°C water bath* for approximately 1 to 2 minutes until no more than two ice crystals remain. **Rapid thawing in this manner is essential to minimise damage to the cells; DO NOT thaw vials in an incubator or your hand**
3. Before opening it wipe the entire vial with a tissue soaked in 70% alcohol
4. Open the lid and pipette the whole content of the vial into a sterile tube (for example 15ml capacity). Then slowly add 5ml of an appropriate pre-warmed medium that has already been supplemented with the required constituents
5. Count the viable cells to ensure the correct seeding density. This can be done microscopically using a haemocytometer and trypan blue stain (the non-viable cells will be stained blue) although other methods are available. For a method and a cell counting calculator you can download go to www.phe-culturecollections.org.uk/cellcounting
6. Check whether the cells are **adherent** or **suspension** cells (if you are unsure check the 'growth mode' in the product detail pages on our website).

i) For **adherent cells** refer to the cell line data on our website for the recommended cell seeding density, i.e. viable cells/cm², then calculate the amount of medium required† and flask size necessary to achieve this.

† For *adherent cells the following culture medium volume ranges (minimum – maximum) are recommended for flask sizes: 25 cm² flask 5-10ml; 75 cm² flask 25–35ml; 175 cm² flask 40-50ml.*

Check the cell line specific data to determine whether the cells require a pre-centrifugation step; adherent cells do not normally require this. However, if the cells are to be used immediately (for example for a cell based assay) a pre-centrifugation step may be advisable to remove residual cryoprotectant. Centrifugation should be at 100 - 150 x g for 5 minutes then re-suspend the pellet in fresh medium using the appropriate volume to achieve the correct seeding density

ii) A pre-centrifugation step is recommended for **suspension cells** to remove cryoprotectant. Centrifuge at 100 - 150 x g for 5 minutes then re-suspend the pellet in fresh medium using the appropriate volume to achieve the correct seeding density i.e. viable cells/ml. We recommend seeding your suspension cells at a relatively high density of 5-7 x 10⁵ cells/ml

7. Incubate the cells at the temperature and CO₂ level recommended on the product detail page. Use flasks with vented caps to allow gaseous exchange if you are using a CO₂ incubator and if CO₂ is required to grow the cells

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. Following resuscitation, seed at $4-5 \times 10^5$ cells/ml. Observe after 24 hours and monitor daily until the cell density has reached $8-9 \times 10^5$ cells/ml before subculturing. Centrifuge at 100 – 150 x g for 5 minutes then re-suspend the cells in fresh medium rather than diluting them.

Often hybridoma cultures can benefit from being re-suspended with media supplemented with 20% foetal bovine serum (FBS) in the early critical stage of culture establishment immediately post resuscitation.

Handling growing cell cultures

Growing cultures can be provided for most cell lines and are particularly valuable for people with less experience of growing cells or for cell lines that are known to be difficult to resuscitate. They are provided in plastic flasks in transport medium that does not contain any antibiotics.

Growing cell cultures should be checked on receipt using an inverted microscope. If you have any problems or queries contact us at: culturecollections.technical@phe.gov.uk or **+44 (0)1980 612684**

Check the cell density. Immediately check the cells upon arrival for any obvious defects by using an inverted microscope. Lay the flask flat in an incubator under the conditions described in the cell line data and incubate overnight. Alternatively, if the cell density is too high (more than 80% confluent) subculture the cells (harvest and reseed) immediately. Follow the guidance for adherent or suspension cells below:

Adherent cells

1. After overnight incubation, remove most of the transport medium – leaving sufficient to just cover the cells. Incubate under the correct conditions as described in the cell line data until the required degree of confluence is achieved
2. Once 80% confluence is achieved, *unless otherwise specified*, carefully remove the culture medium, wash the monolayer twice with phosphate buffered saline (PBS) then add 1-2ml of 0.25% Trypsin/EDTA solution ensuring the cells are covered – decant excess Trypsin/EDTA immediately. **Warning: some cell lines are damaged by trypsin**
3. Incubate at the temperature specified until the cells start to detach from the flask – normally 2-10 minutes
4. Pre-warm fresh medium to the correct incubation temperature for the cells then add 5ml to re-suspend the cells and inactivate the trypsin
5. Count the viable cells. This can be done microscopically using a haemocytometer and trypan blue stain (the non-viable cells will be stained blue) although other methods are available. Calculate the amount of medium required and flask size necessary to achieve the required cell density. For examples of calculations and methods go to: www.phe-culturecollections.org.uk/cellcounting

Suspension cells

1. Following overnight incubation, determine the viable cell density. This can be done microscopically using a haemocytometer and trypan blue stain (the non-viable cells will be stained blue) although other methods are available (go to www.phe-culturecollections.org.uk/cellcounting).
2. Please refer to the cell line data sheet for the correct subculture protocol

Procedure for freezing your cells

Whether you have received frozen or growing cells, ECACC experts advise you to prepare your own frozen stock of the cell line as soon as possible after receipt. *Use aseptic techniques to ensure that you do not contaminate your cells.*

1. Harvest the cells in the log phase of growth. For adherent cells harvest as close to 85% confluency as possible.
2. Check whether there are any special requirements for freezing the cell line. We recommend freezing cells in a cryopreservation solution consisting of 90% serum with 10% dimethyl sulfoxide (DMSO). For some cell types DMSO may not be suitable. If DMSO is not suitable, an alternative, such as glycerol, is specified in the cell line data on the product detail pages.
3. Centrifuge the cells at 150 x *g* for five minutes to create a pellet. Re-suspend the pellet in the cryopreservation solution to give a final cell concentration of 3-5x10⁶ cells per ml for adherent cells and between 6-8 x10⁶ cells per ml. Pipette 1ml aliquots into the plastic vials to be used for storage (preferably internally threaded and with an O-ring though this is dependent on your inventory system).
4. Freeze the cells at a cooling rate of 1-3°C per minute, ideally using a programmable rate-controlled freezer. When the temperature reaches at least -130°C transfer the vials to a gas phase liquid nitrogen storage vessel. If you do not have a programmable rate controlled freezer you can use a polystyrene box or a vessel designed for slow freezing of cultures (such as a Mr Frosty) in a -80°C freezer for a maximum of 24 hours prior to transfer to gaseous phase liquid nitrogen. **Advisory note:** Test cell viability by thawing one vial after short term storage in gas phase liquid nitrogen.

Certificate of Analysis

Certificates of Analysis (CoA) are available via the Culture Collections website. Search for the cell line you want the certificate for, open the correct catalogue entry and click the CoA icon situated in the box on the right hand side of the catalogue entry.

Enter the lot number into the search field for the format of the cells received (usually frozen) and click search. View or download the certificate; if the CoA required is not available via the website then you can request this using the Request Certificate of Analysis link.

For more information on how to access CoAs please use the link in useful references below.

Useful references

Cell culture protocols: www.phe-culturecollections.org.uk/technical

Cell culture reagents: www.phe-culturecollections.org.uk/technical

Glossary: www.phe-culturecollections.org.uk/glossary

Customer service policy: www.phe-culturecollections.org.uk/customerservicepolicy

Technical Support

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