Bioscience Solutions

Lonza

Cell Culture Tips for Cell Lines and Primary Cells Prior to Transfection

Technical Reference Guide

Introduction

In order to help ensuring that your cell lines are in the best possible condition before transfection, please take a moment to review the suggestions below. These are in no way intended to replace product protocols, but rather to give you some helpful hints to facilitate the success of your experiments.

An Important Note About Cancer Cells and Nucleofection™

For the Nucleofection™ of cells from solid tumors, a primary cell kit can be used if the cells are less than passage 3. If the cells are passage 3 or higher, we recommend using a Cell Line Nucleofector™ Kit. For suspension cells, like leukemic cells, if the cells are less than passage 5, a Nucleofector™ Kit for primary cells can be used. If the cells are passage 5 or higher, we recommend using a Cell Line Nucleofector™ Kit.

Passage Number

Cells of a lower passage number typically respond better to transfection and will have higher transfection efficiencies and viabilities than those of higher passage numbers. For the most efficient gene transfer, we recommend using cells that are in logarithmic growth phase and at a passage number lower or less than 10 – 15 (from the time of thaw). This is because some cell lines differentiate and change their features after many passages. If you are going to be transfecting primary cells or cell lines that have been cryopreserved, we recommend that they be passaged at least 2 times to allow them growing properly prior to transfection. Frozen primary blood cells should be incubated in growth medium for a minimum of 1 – 2 hours before transfection.

Growth Conditions

Adherent Cells

For the transfection of adherent cells, the cells should be grown to a certain confluency. In general, the confluency level should be 70 – 85%. The confluency of the culture prior to transfection is important. If the cells are allowed to grow to a higher confluency than recommended, or up to 100% confluency, you may get transfection results other than mentioned in the Optimized Protocols. We also suggest that cells be passaged 2 – 4 days prior to transfection, so that they achieve the required confluency for your experiments. For Nucleofection™, adherent cells must be released from the culture vessel before they can be transfected. Nucleofection™ cannot be performed in culture plates; the cells must be placed into an Amaxa™ Certified Cuvette.

Suspension Cells

Suspension cells should be transfected when they are in the logarithmic growth phase. Generally, this corresponds to a density of 2 – 5 x 10^5 cells per ml. For Nucleofection™ of some cell types, a higher density is recommended. Please check the Optimized Protocol for the cell type you are using. The cells should be passaged 2 – 4 days prior to transfection so that they achieve the required density for your experiments.

For both adherent and suspension cells, it is important to make sure that the culture is growing properly and that the cells have the proper morphology. If they do not, this could indicate contamination with, for example, bacteria, fungi, or mycoplasma. Mycoplasma are common contaminants of cells grown in culture. Studies indicate that between 5 and 35% of cultures are contaminated. Infections, which lead to many serious alterations of cell function and gene expression, are persistent and difficult to detect using conventional methods. We recommend Lonza's inexpensive 20-minute luminescent mycoplasma test – MycoAlert™ Mycoplasma Detection Kit (Cat. No. LT07-118). If mycoplasma are detected, our strong recommendation is to discard the cells. If the cells are truly irreplaceable, MycoZap™ Mycoplasma Elimination Reagent is more gently effective than other treatments (Cat. No. LT07-818). For more information, please visit www.lonza.com/mycoalert.

Cell Harvesting

Proper cell handling during the harvesting process is crucial in order to maintain the health of the cell and helps ensuring the success of your experiments. Before harvesting your cells, we recommend washing the monolayer to get rid of any residual growth medium, as well as calcium and magnesium ions. In most cases, PBS or HBSS without calcium or magnesium can be used. Other wash solutions can be used as well and will depend on the characteristics of the cells in use. For example, cultures that have multiple layers may detach easier if they are washed first with a 0.5 mM – 1 mM EDTA solution or trypsin solution.

For Nucleofection™, adherent cells will need to be detached from the culture vessel before they can be transfected. For cell lines, we recommend using trypsin at a concentration of 0.05% (0.5 mg/ml) and EDTA at 0.48 mM (0.2 mg/ml) in a balanced salt solution without calcium and magnesium.

Primary cells, like Clonetics™ Cells, should be treated more gently than cell lines. For example, we recommend using Clonetics™ ReagentPack™ (CC-5034), which contains a gentle trypsin solution, HEPES buffered saline, and trypsin neutralizing solution.

Other dissociative enzymes can be used and again, this depends on the characteristics of the cells. For example, collagen rich cultures may require a collagenase dissociation. Please check with the cell supplier for specific recommendations.

Once the cells have detached from the growth vessel, inactivate the trypsin by adding either:

- Growth medium with serum
- Trypsin neutralizing solution from ReagentPack™ (CC-5034)
- PBS/0.5% BSA

When using biochemical transfection reagents (e.g., HiFect™ Transfection Reagent) for transfection, it is not necessary to de-tach the cells from the growth vessel.

In all cases, it is important to monitor the cells during trypsin treatment because, if the trypsin is allowed to remain on the cells longer than necessary, it will damage the cell membranes resulting in high cell mortality.

Do not scrape the monolayer unless specifically recommended by the cell supplier. Scraping can cause mechanical damage to the cells and will not result in a single cell suspension.

When working with suspension cells, no detachment is necessary, so you only need to spin down the required number of cells and remove as much residual growth medium as possible from the cell pellet. For Nucleofection™, the cells will then be resuspended in Nucleofector™ Solution.

It is also important to avoid extra pipetting or unnecessary washing steps. Do not vortex your cells, as extra handling beyond what is recommended can potentially harm the cells and result in high cell mortality.

Helpful Hints for Adherent Cells

If you are working with a strongly adherent cell line, you can use a stronger trypsin solution. Solutions of 0.25% and 0.5% trypsin are routinely available from commercial suppliers. Alternatively, instead of washing the monolayer with PBS before the addition of trypsin, you can use a trypsin solution as the wash. Aspirate the trypsin, replace with fresh trypsin solution, and incubate until the cells detach.

If the cells are weakly adherent, you can wash with EDTA alone, which may be enough to detach the cells or you can try the following:

- Wash with PBS
- Add trypsin and immediately aspirate off the trypsin solution
- Incubate the cells with the residual solution until they detach

Alternatively, you can also wash with ice cold PBS if the cells are particularly loose. In many cases, this alone may cause them to detach.

An Important Note About Lipids and Transfection

Most lipids cannot be used on suspension cells. It is also important not to add antibiotics to the medium during transfection with lipids, as this will cause cell death. Antibiotics can be added to the growth medium after transfection. In some cases, it may be helpful to allow the cells to recover for 12 – 24 hours before adding in any antibiotics. Serum can be present in the growth medium after transfection and, in some cases, it can also be present in the transfection medium. Serum must not be present during complex formation because serum inhibits the formation of the liposome complexes. With lipids, it is also possible to scale up the transfection by varying the amounts of lipid, DNA, cells, and medium in proportion to the relative surface area of the culture vessel. Please check the protocol specific to your lipid for guidelines.

An Important Note About Centrifugation

For Nucleofection™, it is important to follow the centrifugation guidelines as stated in the Optimized Protocols. Our standard for centrifugation is 90 xg. We do not use RPM's because the speed you select in order to achieve 90 xg will vary with the type of rotor in use in your lab. In order to determine the required speed to get 90 xg, please consult the operation manual for your centrifuge or rotor.

If you do not have the manual, please visit the following link for a nomogram that you can use to convert g-forces to RPM: https://www.aphl.org/programs/infectious_disease/tuberculosis/TBCore/Nomogram.pdf

Alternatively, the correct rotor speed can be calculated by using the converter found here: https://druckerdiagnostics.com/g-force-calculator/

Centrifugation speeds and g-forces are not as critical with other transfection methods (i.e., standard electroporation, lipids) as they are with Nucleofection™, which is why you will not see specific guidelines given on many protocols.

Cell Sources

For best transfection results, we recommend the use of cells with a known history, i.e., low passage number and free from contamination. For primary cells, we recommend using Clonetics™ and Poietics™ Cells from Lonza.

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