BioResearch

Lonza

Transient Protein Production Using Nucleofector™ Technology
Technical Reference Guide

1. Background

Transient protein expression in mammalian and insect cell lines has gained increasing relevance, enabling fast and flexible production of high quality eukaryotic protein. Protein quantities of up to 10 mg can be produced within several days, meaning a significant shortening of process time in comparison to protein production from stable clones where generation of producer cells and production of protein can take up to several months. Suspension CHO (sCHO), HEK293 (sHEK), NSO cells, and insect cells, for example, are ideal target cell lines for transient protein production as the cells can be cultured in high density and under serum-free conditions. However, many individual sCHO and sHEK293 clones, as well as NSO and insect cells, are difficult to transfect using conventional transfection methods. This means that transfection of these suspension cell lines using conventional methods yields efficiencies that are mediocre at best, resulting in low transient protein production. The Nucleofector™ Technology helps circumventing this problem because it achieves efficient gene transfer even into difficult-to-transfect cell lines like individual sCHO or sHEK293 clones or other cell lines relevant for protein production.

This Technical Reference Guide provides an experimental outline for transient protein production in a 30 ml volume scale using suspension HEK293 or CHO in the Nucleofector™ I/II/2b Device. For transient protein expression experiments using other cell types or other Nucleofector™ Platforms, e.g. the 4D-Nucleofector™ System, please contact Lonza Scientific Support at scientific.support@lonza.com

2. Experimental Outline for 30 ml Batch Using Suspension HEK293 or CHO

2.1 Preparation and Quality

As recommended by cell supplier, suspension CHO and HEK293 clones should be grown in a shaking flask with a vented cap [e.g., VWR polysterene shaking flask 125 ml, baffled base, VWR Cat. No. 30180-040], to a density of 10^6 cells/ml in 30 ml medium (= 3 x 10^7 cells in total).

2.2 DNA Preparation and Quality

The quality and the concentration of DNA used for Nucleofection™ plays a central role in the efficiency of gene transfer. We strongly recommend the use of high quality products for plasmid purifi cation like EndoFree® Plasmid Kits [QIAGEN, Cat. No. 12391 Giga Kit, 12362 Maxi Kit, 12381 Mega Kit]. The purified DNA should be resuspended in deionized water or TE buffer (10 mM Tris/HCl, 1 mM EDTA, pH 8.0) with a concentration between 0.2 – 1 µg/µl. Please check the purity of each plasmid preparation by measurement of the A260:A280 ratio, according to QIAGEN protocol.

2.3 Important Vector Information

The pSPORT vector family [Invitrogen, pCMV SPORT6] which includes a CMV promoter, typically works well for transient protein expression in HEK293 and CHO.

In HEK293E (EBNA) cells, the use of vectors containing EBV oriP can further boost the transient expression yield.

In HEK293T cells, the use of vectors containing the SV40 ori can further boost the transient expression yield.

We do not recommend usage of IRES sequences, since the gene encoded 3' of the IRES sequence is usually expressed to a lesser extent than the upstream gene, and in some experimental conditions may not be expressed at all. As alternatives, we suggest either co-transfecting 2 (or more) plasmids, using 1 plasmid with each gene under the control of its own promoter, or making a fusion protein.

2.4 Nucleofection™ Protocol

Preparation of Culture Flask

Pre-fill sterile shaking flask (with vented cap, e.g., VWR polysterene shaking flask 125 ml, baffled base) with 10 ml prewarmed medium recommended by supplier of cells.

Preparation of Nucleofector™ Solution

Add 0.5 ml Supplement to 2.25 ml Nucleofector™ Solution and mix gently. The Nucleofector™ Solution is now ready to use and is stable for 3 months at 4°C. Note the date on the vial.

Setup of Nucleofection™ for 30 ml Batch

Prepare one mixture for 10 separate Nucleofection™ Samples: 3 x 10^7 cells; 20 – 100 µg highly purified plasmid DNA, (in 10 – 20 µl H_2O or TE); 1 ml Nucleofector™ Solution.

Note

Since the concentration of plasmid DNA has a strong effect on transient protein yields, it is recommended to test varying amounts of plasmid DNA between 2 and 10 µg/Nucleofection™ Sample for optimization of protein expression rates.

Cell Type-specific Conditions

CHO-S cells, SFM adapted (Invitrogen, Cat.No. 11619012): Cell Line Nucleofector™ Kit V, Program U-024, culture medium and conditions as recommended by supplier.

293 F cells, SFM adapted (Invitrogen, Cat.No. 11625019): Cell Line Nucleofector™ Kit V, Program X-001, culture medium and conditions as recommended by supplier.

For other cell clones, check Lonza's online cell database at **www.lonza.com/cell-database**.

Preparation of Samples

1. Cultivate cells to 3 x 10^7 cells in total as required for a 30 ml batch volume incubation.
2. Centrifuge the cell suspension (3 x 10^7 cells) at 100 xg for 8 min. Discard supernatant completely so that no residual medium covers the cell pellet.
3. Resuspend the pellet in 1.0 ml room temperature Nucleofector™ Solution V to a final concentration of 3 x 10^6 cells/100 µl.

Note

Avoid storing the cell suspension longer than 15 min in Nucleofector™ Solution as this reduces cell viability and gene transfer efficiency.

Nucleofection™

4. Mix cell suspension with 20 – 100 µg DNA (in 10 – 20 µl H_2O or TE).
5. Transfer 110 µl of the Nucleofection™ Sample into a Certified Cuvette. Make sure that the sample covers the bottom of the cuvette. Avoid air bubbles while pipetting. Close the cuvette with the blue cap.
6. Select the appropriate Nucleofector™ Program (please find program codes in section 2.4). Insert the cuvette into the cuvette holder (Nucleofector™ I Device: rotate carousel to final position) and press the "X" button to start the program.
7. Take the cuvette out of the holder. Using the plastic pipettes provided in the kit, dilute Nucleofection™ Mixture in cuvette with approx. 500 µl prewarmed medium and transfer suspension to shaking flask (e.g., VWR polysterene shaking flask 125 ml, vented cap, baffled base) pre-filled with 10 ml prewarmed medium.

Note

To avoid damage to the cells, add pre-warmed medium and remove suspension from the cuvette immediately after the program has finished (display showing "OK").

Incubation for Protein Production Post Nucleofection™

8. Press the "X" button to reset the Nucleofector™ Device.
9. Repeat steps 5 – 8 for the remaining samples.
10. Add pre-warmed medium to the shaking flask to a final volume of 30 ml.
11. Return shaking flask to an orbital shaker platform at approximately 100 – 150 rpm in an incubator at 37°C according to the supplier of cells (for exact shaker speed and other culture details refer to information provided by cell supplier).
12. Collect samples at the desired time points for protein expression analysis. In first pilot studies, samples should be taken every 24 hours to evaluate best time point for harvesting.

3. Parameters for Optimization

Concentration of Plasmid
Test varying amounts of plasmid from 2 – 10 µg/Nucleofection™ Reaction in order to find the optimal concentration for transient protein expression.

Gassing/Ventilation
Check ventilation of cell culture in incubator and flask (shaking speed, volume of suspension per bottle, gas permeability of cap).

Time Point for Harvest
When expressing a protein for the first time, optimal time point for harvest needs to be evaluated by performing a time-course experiment between days 1 and 7 post Nucleofection™ to identify the peak of protein production and to monitor cell viability. Protein expression is often detectable at 4 – 8 hours post Nucleofection™, peak yields for IgG protein production have been observed at 5 to 7 days post Nucleofection™ .

Vector Type
Promotors should be checked that it is appropriate for the cell type used. Vector construct should be checked that it is appropriate for protein of interest and cell type (orientation of tags, solubility or secretion tags, etc).

Cell Type
For some difficult-to-express proteins, testing of alternative cell types might be considered for improvement of protein yield.

For more information on protein expression and production visit **www.lonza.com/protein-production**.

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