Application Note

Efficient and Scalable Protein Expression in Suspension CHO Cells Using the NovaCHOice® Transfection System

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Introduction

Protein expression in mammalian cells is the method of choice for the production of bioactive proteins displaying appropriate post-translational modifications. A convenient approach to obtain bioactive proteins and shorten the evaluation cycles is to use transient transfection. One of the preferred cells systems for production of bioactive proteins is Chinese Hamster Ovary (CHO) cells that have been adapted for growth in suspension. However, suspension CHO (CHO-S) cells tend to be refractory to transfection using many of the commonly-used transfection reagents. Here we present data on the use of the NovaCHOice® transfection system, consisting of a core reagent and an optional booster, to obtain high levels of protein expression in CHO-S cells. Our data indicate that use of NovaCHOice® transfection system results in high transfection efficiency and high levels of protein expression within 24 h. Our data further indicate that by 48 h and 72 h the proportion of high-expressing cells is significantly increased with very low cellular toxicity. In addition, no medium changes are required post-transfection, thus reducing cell losses and hands-on time. In summary, the NovaCHOice® transfection system is well-suited for transient and stable transfections in CHO-S cells, due to its high transfection efficiency and low toxicity.

Materials and Methods

Protein expression in CHO-S cells was evaluated using transfection with plasmids expressing either Green Fluorescent Protein (GFP) or human Secreted Alkaline Phosphatase (SEAP). CHO-S cells were transfected using the NovaCHOice® Transfection Kit in 15 mL cultures in a shaker flask unless otherwise indicated. The day prior to transfection, the cells were passaged at 0.5 x 10^6 cells/mL and diluted to a concentration of 1x10^6 cells/mL the day of transfection.

All cell concentrations were determined using the Scepter™ handheld, automated cell counter. For 15 mL transfection, 15 µg DNA, 15 µL NovaCHOice® Transfection Reagent and 7.5 µL NovaCHOice® Booster Reagent were mixed in 1500 µL OptiPRO™ medium (Life Technologies; 1:1:0.5 ratio). DNA was added into the prepared tube with culture medium, followed by the transfection reagent then the booster reagent. and the mixture was mixed gently and allowed to incubate at room temperature for 15 minutes before it was added dropwise to the flask of cells.

SEAP expression was determined at 24, 48 and 72 h post-transfection by removing 50 µL of the culture at the corresponding time point, centrifuging the cells and collecting the supernatant. The supertantant was kept at -20 °C until assayed. Supernatants were assayed using the SEAPorter™ kit (Imgenex, Cat. No. 10055K).

Expression of GFP was evaluated by fluorescent imaging and by flow cytometry. For fluorescence imaging, 500 µL of the culture was removed at 24, 48 and 72 h post-transfection and plated onto poly-L-lysine-coated 24-well plates. An additional 500 µL of sample was also removed from each flask at 24, 48 and 72 h for flow cytometry analysis.

To determine the scalability of transfection with the NovaCHOice® Transfection Kit, 10 mL, 100 mL and 1000 mL cultures were transfected with SEAP-encoding plasmid DNA (SEAP pDNA) as described above. Culture samples were collected at 24, 48 and 72 h post-transfection.

Results

High levels of SEAP expression (~1,000 ng/mL) was observed 24 h post-transfection with a maximum of (~1,200 ng/mL) after 48 h (Figure 1).

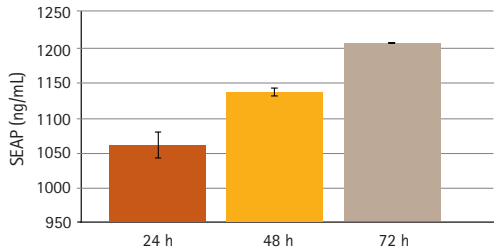

Figure 1. High levels of SEAP expression is evident 24 h post-transfection. Levels of SEAP secreted into the growth medium reached 1050 ng/mL 24 h after transfection; an increase of close to 20% in levels of secreted SEAP were evident 72 h post-transfection. Error bars indicate standard deviation.

Transfection scalability data are shown in Figure 2. SEAP expression levels increased proportionally to volume at all time points. This observation was consistent with the fluorescence imaging and flow cytometric GFP expression data. Fluorescence imaging data (Figure 3) showed that a high proportion of the cells were expressing the GFP marker at 24 h; by 48 h nearly all cells in the field expressed the fluorescent marker. In addition, cell morphology as visualized during imaging indicated low toxicity after transfection.

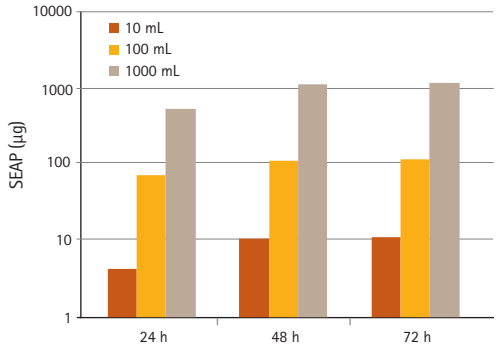

Figure 2. The NovaCHOice® Transfection Kit allows for scalable expression of SEAP in CHO-S systems. Levels of SEAP in the culture medium were determined as described in the Materials and Methods section at the indicated time points. Total SEAP produced at each scale and time point closely followed the 10-fold step increase in culture size.

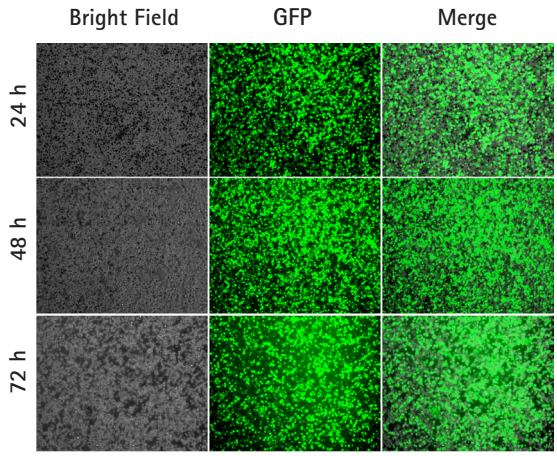

Figure 3. Fluorescence imaging indicates high transfection efficiency. Images were acquired at 24 h, 48 h and 72 h post-transfection using an inverted fluorescent microscope (Zeiss Axiovert 200). Cells expressing GFP were imaged using FITC channel (Ex. 490, Em. 525) and merged with the brightfield image using ImageJ, version 1.4.3.67.

These observations were supported by the flow cytometric analysis (Figure 4), which showed that 90% of the cells were positive for GFP expression 24 h after transfection and close to 99% by 48 h.

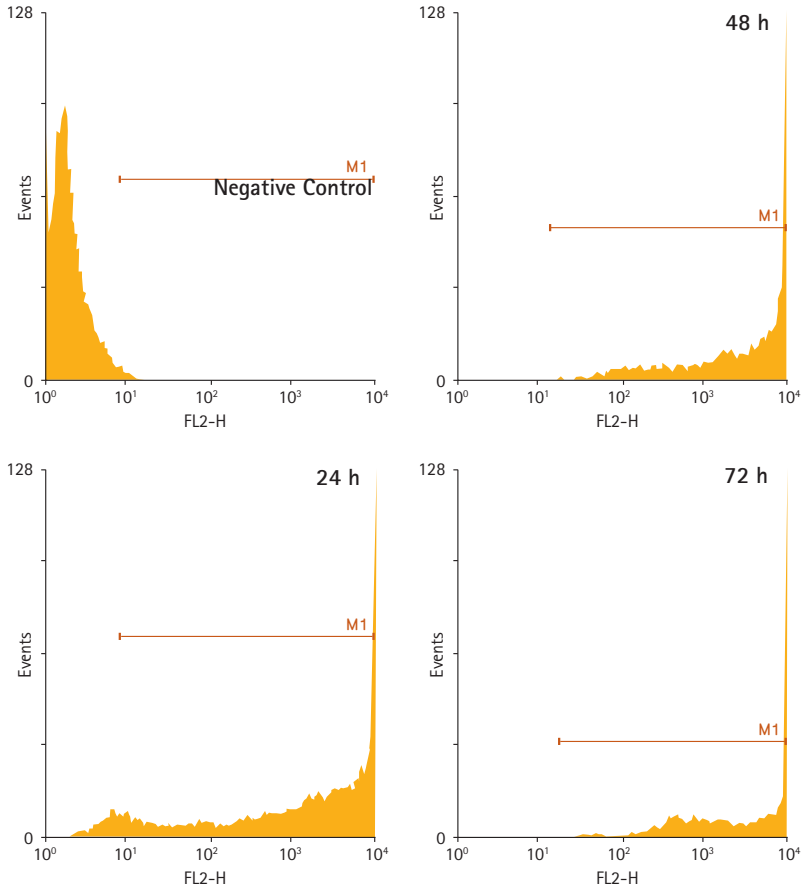

Figure 4. Transfection of CHO-S cells using NovaCHOice® Transfection Kit results in high transfection efficiency. Flow cytometry data for GFP-expressing cells are shown for the time points indicated. A high proportion of cells was positive for GFP expression 24 h post-transfection.

Discussion

The data presented here demonstrate that the NovaCHOice® transfection system is well-suited for rapid, high levels of protein expression in suspension CHO cells. Maximum levels of expression of both secreted (SEAP) and intracellular (GFP) reporters are obtained 24-48 h after transfection. Both fluorescence imaging and flow cytometric analysis indicate that transfection efficiencies approach 99% 48 h post-transfection; in addition, flow cytometric data show a high proportion of high expressing cells. Although only transient transfection data are presented here, it would be expected that the NovaCHOice® transfection system would be attractive as a first step in the production of stably expressing lines due to its high transfection efficiency and low toxicity. Importantly, transfections are scalable from 10 mL to 1 L, which makes the NovaCHOice® transfection system attractive for scientists interested in producing large quantities of protein by transient transfection.

For protein research as well as for the development of large-molecule biotherapeutics, it is crucial to produce and purify sufficient quantities of functionally active, highly pure, recombinant protein. Using the same batch of recombinant protein across all studies can help eliminate equivocal results generated by lot-to-lot variability in protein activity and sample composition. Therefore, it is highly desirable to obtain maximum yield from each batch of cells expressing the recombinant protein.

By enabling researchers to quickly obtain high yields of recombinant protein in CHO-S cells, the NovaCHOice® transfection system has the potential to accelerate protein research and maximize the information obtained from every experiment.

FEATURED PRODUCTS

Available from www.millipore.com.

Description	Catalogue No.
NovaCHOice® Transfection Kit	72622-3 and 72622-4
Scepter™ 2.0 Handheld Automated Cell Counter	PHCC20060

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