Transient Expression of Proteins Using COS Cells

BASIC PROTOCOL

Three factors contribute to make COS cell expression systems appropriate for the high-level, short-term expression of proteins: (1) the high copy number achieved by SV40 origin–containing plasmids in COS cells 48 hr posttransfection, (2) the availability of good COS cell expression/shuttle vectors, and (3) the availability of simple methods for the efficient transfection of COS cells. Each COS cell transfected with DNA encoding a cell-surface antigen (in the appropriate vector) or cytoplasmic protein will express several thousand to several hundred thousand copies of the protein 72 hr posttransfection. If the transfected DNA encodes a secreted protein, up to 10 μg of protein can be recovered from the supernatant of the transfected COS cells 1 week posttransfection. COS cell transient expression systems have also been used to screen cDNA libraries, to isolate cDNAs encoding cell-surface proteins, secreted proteins, and DNA binding proteins, and to test protein expression vectors rapidly prior to the preparation of stable cell lines (*UNIT 9.5*).

This transfection protocol is a modification of that presented in *UNIT 9.2* and gives conditions for optimal transfection of COS cells (also *UNIT 9.9*). The main difference between this procedure and that in *UNIT 9.2* is the composition of the DEAE-dextran/chloroquine solution, which is prepared here in PBS, not TBS, and contains chloroquine to prevent acidification of endosomes presumed to carry DEAE-dextran/DNA into the cell. This acidification results in acid hydrolysis of DNA, giving rise to mutations and destruction of the DNA. With this protocol, 40-70% of the cells can be routinely transfected.

Materials (see APPENDIX 1 for items with ✓)

Appropriate vector (CDM8, pXM, or pDC201; Invitrogen)
COS-7 cells to be transfected (ATCC #CRL1651)
Dulbeccos minimum essential medium with 10% calf serum (DMEM-10 CS)
DMEM with 10% NuSerum (Collaborative Research #55000) (DMEM-10 NS), 37°C
✓ Phosphate-buffered saline (PBS)
DEAE-dextran/chloroquine solution: 10 mg/ml DEAE-dextran (Sigma #D9885) + 2.5 mM chloroquine (Sigma #C6628) in PBS
10% dimethyl sulfoxide (DMSO; Sigma #D5879) in PBS
0.5 mM EDTA in PBS

100-mm tissue culture dishes
Humidified 37°C, 6% CO_2 incubator
Phase-contrast microscope
Sorvall RT-6000B rotor or equivalent

1. Subclone gene of interest into appropriate vector to get desired recombinant DNA (*UNIT 3.16*). Purify recombinant DNA by miniprep procedure (5-ml culture) or by CsCl/EtBr centrifugation (*UNITS 1.6, 1.7 & 9.1*).

2. Split confluent COS-7 cells (~10^6 per 100-mm dish) in DMEM-10 CS at a 1:5 ratio the day prior to transfection so they will be ~50% confluent the next day. Grow cells overnight in a CO_2 incubator (6% CO_2) at 37°C to ~50% confluence.

3. Just before use (for each 100-mm dish of COS cells to be transfected), thoroughly mix 5 ml of 37°C DMEM-10 NS with 5 to 10 µg recombinant DNA and mix. Add 0.2 ml of DEAE-dextran/chloroquine solution.

   It is important that DEAE-dextran be well mixed with media before adding DNA; otherwise, the DNA will form large precipitates with DEAE-dextran. These large precipitates cannot be taken up by the cell, resulting in a reduced transfection efficiency.

4. Aspirate medium from COS cells and for each 100-mm dish, add DMEM-10 CS/DEAE-dextran/DNA prepared in step 2. Incubate cells 3 to 4 hr (may require optimization) in a CO_2 incubator at 37°C. Observe cells using phase-contrast microscope.

   The DEAE-dextran will cause cells to retract and become vacuolated. Efficiency of transfection increases with longer incubation periods; on the other hand, so does cell death.

5. Aspirate DMEM/DEAE-dextran/DNA and add 5 ml of 10% DMSO (prepared in PBS). Incubate cells 2 min at room temperature. Aspirate DMSO and add 10 ml DMEM-10 CS. Grow cells overnight (12 to 20 hr) in CO_2 incubator at 37°C.

6. Passage each 100-mm dish of transfected COS cells into two new 100-mm dishes. Grow cells at 37°C as in step 7a or 7b.

7a. When expressing secreted proteins, add 5 ml DMEM-10 CS 96 hr (4 days) after completing step 6 and incubate 4 days. Harvest medium, remove dead cells and debris by centrifuging 10 min at ~1000 × *g* (~2000 rpm in Sorvall RT-6000B rotor), room temperature, and save supernatant. Detect secreted proteins by metabolic labeling (*UNIT 10.18*) and immunoprecipitation (*UNIT 10.16*), immunoaffinity chromatography (*UNIT 10.11*), radioimmunoassay (*UNIT 11.16*), western blotting (*UNIT 10.8*), or bioassay (*UNIT 9.5*).

   Do not aspirate the old medium prior to addition of 5 ml DMEM-10 CS because this medium contains secreted protein. Addition of extra medium 96 hr posttransfection results in better yield of expressed protein; however, it also increases level of total protein since medium contains 10% serum. To eliminate this problem, COS cells can be placed in serum-free medium 10 to 12 hr after they have been replated although this may result in a 10-fold lower yield of expressed protein than in the presence of serum.

7b. When expressing cell-surface or intracellular proteins, aspirate medium from cells 72 hr after transfection in step 5. Add 5 ml PBS, swirl, and aspirate PBS. Add 5 ml of 0.5 mM EDTA in PBS and incubate 15 min in CO_2 incubator at 37°C. Lift cells from dish by gently dislodging with a Pasteur pipet. Stain cell-surface proteins with appropriate fluorescent antibody and detect by microscopy or flow cytometry.

Transfected COS cells will tend to clump when lifted from the dish. Pipetting the cells up and down will tend to disrupt these clumps. More effective dispersion of the clumps can be obtained by forcing the cells through a 100-μM nylon mesh.

Reference: Warren and Shields, 1984.

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Amplification Using CHO Cell Expression Vectors

The ability to select for integration of plasmid DNA into the host chromosome allows the generation of stably transfected cell lines. With transfection of a selectable marker linked to a nonselectable target gene (or cotransfection of the two unlinked genes), high-level expression of the desired gene is obtained by selecting for amplification of the selectable marker. The first basic protocol describes the dihydrofolate reductase (DHFR) selection system while the second is based on selection of the glutamine synthetase (GS) gene. The DHFR system is probably more widely used, and results in very high levels of amplification (up to 1000 copies per cell in some cases) and expression; however, the amplification process is lengthy and can require from 9 to 12 months to isolate a stable, amplified line. In contrast, the GS typically requires only a single round of selection for amplification to achieve maximal expression levels; in this system the time required to isolate stably amplified clones from primary transfectants is dramatically reduced to 2 months or less.

Cell lines containing at least 1000 copies of an exogenous gene can be produced using the DHFR selection system. The levels of mRNA and protein obtained depend upon the target gene to be expressed, but can constitute up to 5% of total protein synthesis. Introduction of pEE14-based vectors using $CaPO_4$-mediated transfection usually leads to multiple copies of the vector becoming integrated in the genome in the GS selection system. The copy number can increase up to 30-fold in one round of selection for amplification. The amount of product depends both on the individual transfectant and on the protein being expresssed, but can be up to 10 μg protein/10^6 cells per 24-hr period for some proteins. On amplification, protein expression can parallel the increase in copy number but is likely to reach a plateau, usually after a single round of amplification for many secreted proteins, probably because the secretion apparatus is saturated.

AMPLIFICATION USING DIHYDROFOLATE REDUCTASE

BASIC PROTOCOL

The pED series of dicistronic vectors (Fig. 16.14.1) can be used to obtain high-level expression of a targeted gene in stably transfected cells. These vectors carry a cloning sequence for insertion of the target gene followed by the selectable and amplifiable marker gene, dihydrofolate reductase (DHFR). Alternatively, a plasmid expressing the gene of interest and a plasmid expressing DHFR can be cotransfected. DHFR-deficient CHO cells