# Complete Control Cell Lines

## INSTRUCTION MANUAL

Catalog #222100, #222105, and #222110

Revision A

**For In Vitro Use Only**

222100-12

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# Complete Control Cell Lines

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# Complete Control Cell Lines

## MATERIALS PROVIDED

| Catalog # | Material provided | Quantity | Storage condition[a] |
|---|---|---|---|
| #222100 | ER-CHO cell line | 1-ml cryovial (~1 x $10^6$ cells) | Liquid nitrogen |
| #222105 | ER-NIH cell line | 1-ml cryovial (~1 x $10^6$ cells) | Liquid nitrogen |
| #222110 | ER-293 cell line | 1-ml cryovial (~1 x $10^6$ cells) | Liquid nitrogen |

[a] Immediately on receipt, store the cryovial of cells in liquid nitrogen. Protective gloves, clothing, and a face mask or goggles should be worn when transferring the cryovial to and from liquid nitrogen.

## STORAGE CONDITIONS

**Store cryovials immediately in liquid nitrogen**

## ADDITIONAL MATERIALS REQUIRED

Liquid nitrogen storage tank
Growth medium[§]
Freezing medium[§]
G418
Trypsin–EDTA[§]
Phosphate-buffered saline (PBS)[§]
Plasticware
Incubator (5–7% $CO_2$, 37°C)
Controlled-freezing container (e.g., the StrataCooler Cryo preservation module, the StrataCooler Cryo Lite preservation module, or the equivalent)
Tabletop centrifuge

[§] See *Preparation of Media and Reagents.*

Revision A

# NOTICES TO PURCHASER

## CMV Promoter

The use of the CMV Promoter is covered under U.S. Patent Nos. 5,168,062 and 5,385,839 owned by the University of Iowa Research Foundation and licensed FOR RESEARCH USE ONLY. For further information, please contact UIRF at 319-335-4546.

## pERV3 Translation Enhancer

Use of the translation enhancer of the pERV3 Vector and the Complete Control vector kit is covered by U.S. Patent No. 4,937,190 and is limited to use solely for research purposes. Any other use of the translation enhancer of the pERV3 Vector and the Complete Control vector kit requires a license from WARF.

## Complete Control Mammalian Expression System

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# INTRODUCTION

The Complete Control cell lines are part of the Complete Control inducible mammalian expression system, a system that allows precise control of gene expression. Two vectors are required to express a gene of interest: the pEGSH expression vector and the pERV3 receptor vector. The pEGSH expression vector has a site for inserting the gene of interest and an inducible promoter regulated by ponasterone A (ponA), an analog of the insect hormone ecdysone. The pERV3 receptor vector (see Figure 1) contains an expression cassette for the receptors VgEcR and RXR, which are required for ponA-induced transactivation of the pEGSH vector.

Most applications of the Complete Control system are based on the stable integration of the pERV3 and pEGSH vectors into the genome of a cell line. These applications require double-stable cell lines that have all of the following: optimal receptor expression, the inducible expression cassette inserted into a site in a chromosome that is readily accessible to the receptors, and low-level uninduced expression of the gene of interest. Cotransfecting the pERV3 and pEGSH vectors and screening the cells in a single round is not an effective method for producing such a double-stable cell line. The most effective method is to first integrate the pERV3 vector into a cell line, screen the resulting cell lines to choose the cell line with optimal receptor-expression (using transient transfection of the inducible pEGSH-Luc vector), and then stably transfect the pEGSH vector into the chosen cell line.

Because two rounds of stable cell screening is tedious, we have engineered three Complete Control cell lines that eliminate the need to produce and screen stable pERV receptor-expressing cell lines. The three cell lines, ER-CHO, ER-NIH, and ER-293, are derived from Chinese hamster ovary cells, mouse fibroblast cells, and human primary embryonal kidney cells, respectively. The Complete Control cell lines stably express optimal levels of the VgEcR and RXR receptors. In transient expression assays with the pEGSH-Luc vector, they show a high level of ponA-dependent transactivation; a pEGSH-Luc double-stable cell line, derived from ER-CHO, has been shown to be induced 1,000-fold.[1] Use of these cell lines for preliminary determination of whether expression of a gene of interest is appropriately controlled eliminates the need to optimize the amount of pERV3 vector to be cotransfected with the pEGSH construct into target cells.

## The pERV3 Vector

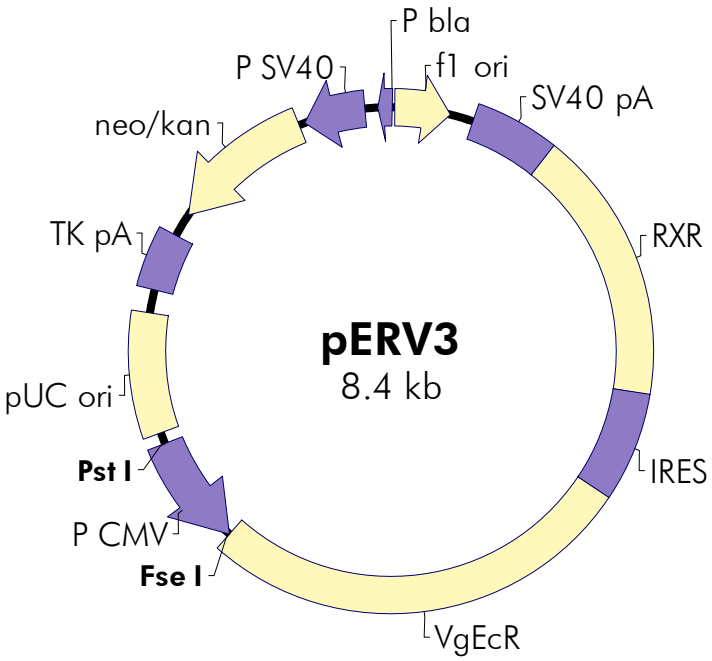


**FIGURE 1** Map of the pERV3 vector. The pERV3 vector contains an expression cassette from which a dicistronic message encoding the VgEcR and RXR receptors is transcribed from the CMV promoter. It is possible to replace the CMV promoter with a promoter of interest using *Pst*I and *Fse*I. The RXR protein expressed in this system is essentially wild-type. The internal ribosome entry site (IRES), which is derived from the encephalomyocarditis virus (EMCV) 5'UTR, allows efficient internal initiation of translation of the RXR receptor. The plasmid contains the neomycin-resistance gene downstream of both the SV40 early promoter and the β-lactamase promoter to allow selection of mammalian cell transfectants (with G418) and *Escherichia coli* transformants (with kanamycin).

# CELL CULTURE PROTOCOLS

## Starting the Cells in Culture

1. Prepare the growth medium[§] and transfer 10 ml of the growth medium to a 15-ml conical tube.

2. Thaw the frozen cryovial of cells within 40–60 seconds by rapid agitation in a 37°C water bath. Remove the cryovial from the water and immediately immerse the cryovial in 70% (v/v) ethanol at room temperature.

   **Note** *All procedures from this point should be carried out using sterile technique.*

3. Transfer the cell suspension to the conical tube containing the growth medium.

4. Centrifuge the cells at 200 × *g* for 5 minutes at room temperature and remove the growth medium from the conical tube by aspiration.

5. Prepare and add 10 ml of growth medium containing G418 (500 µg/ml for ER-CHO cells or 300 µg/ml for ER-NIH and ER-293 cells) to a 75-$cm^2$ tissue culture flask. Resuspend the cells in the conical tube in 5 ml of growth medium containing G418. Transfer the cell suspension to the tissue culture flask.

6. Place the cells in a 37°C incubator with 5–7% $CO_2$.

## Passaging the Cells

**Note** *Split the cells when the cell monolayer is ~80–90% confluent or approximately every 3–4 days.*

1. Remove the medium by aspiration, wash the cells with PBS,[§] and then remove the PBS by aspiration.

2. Add 1.5 ml of trypsin–EDTA[§] to each 75-$cm^2$ tissue culture flask. Incubate the cells at room temperature for ~2–5 minutes. Tap the flask to release the adherent cells.

   **Note** *Incubate the cells in the trypsin–EDTA for the minimum time necessary for the adherent cells to release from the flask. Overtrypsinization can kill the cells.*

[§] See *Preparation of Media and Reagents.*

3. Dilute the cells with growth medium containing G418 to a final volume of 5–10 ml. The serum in the medium inactivates the trypsin. Transfer the cells to the desired number of 75-$cm^2$ or 175-$cm^2$ tissue culture flasks. Depending on the desired use for the cells, use an inoculum of $1 \times 10^4$ to $1 \times 10^6$ cells. Add growth medium to the flasks to a final volume of 15 ml for 75-$cm^2$ tissue culture flasks or to a final volume of 30 ml for 175-$cm^2$ tissue culture flasks.

4. Place the cells in a 37°C incubator with 5–7% $CO_2$.

## Freezing the Cells for Long-Term Storage

1. Remove the medium by aspiration, wash the cells with PBS, and then remove the PBS by aspiration.

2. Add 1.5 ml of trypsin–EDTA to each 75-$cm^2$ tissue culture flask (or 2 ml/175-$cm^2$ flask). Incubate the cells at room temperature for ~2–5 minutes. Tap the flask to release the adherent cells.

   ***Note*** *Incubate the cells in the trypsin–EDTA for the minimum time necessary for the adherent cells to release from the flask. Overtrypsinization can kill the cells.*

3. Dilute the cells with 8.5 ml of growth medium. The serum in the medium inactivates the trypsin.

4. Transfer the cell suspension to a 15-ml conical tube. Centrifuge the cells at 200 × *g* for 5 minutes at room temperature. Remove the growth medium from the tube by aspiration.

5. Resuspend the cells in 1–2 ml of freezing medium (see *Preparation of Media and Reagents*).

6. Transfer the cells to cryovials and place the cryovials in a chilled controlled-freezing container. Incubate the cryovials at –80°C overnight.

7. The following day, transfer the cryovials to liquid nitrogen for long-term storage. If properly stored, the cells should remain stable for years.

## Transfection with the pEGSH Vector

The Complete Control cell lines can now be passaged for transfection with a pEGSH construct containing your gene of interest. Please see the *Complete Control Inducible Mammalian Expression System Instruction Manual* for detailed instructions.

## TROUBLESHOOTING

| Observation | Suggestion(s) |
|---|---|
| Cells do not survive resuspension in growth medium following storage in liquid nitrogen | Frozen cells are very susceptible to freeze thawing and fluctuations in temperature. Store the cryovials of cells in liquid nitrogen immediately on receipt to avoid problems associated with incorrect |
| | Thaw the cells quickly and immediately dilute the cells in the growth medium to avoid problems associated with incorrect thawing |
| Cells do not survive passaging | When passaging, inoculate a fresh tissue culture flask with $1 \times 10^4$ to $1 \times 10^6$ cells |
| | Incubate the cells with trypsin–EDTA for the minimum time necessary for the cells to release from the flask |
| | Split the cells when the monolayer of cells is at most 90% confluent |

## PREPARATION OF MEDIA AND REAGENTS

### Freezing Medium

9 ml of fetal bovine serum
1 ml of dimethylsulfoxide (DMSO)
Filter sterilize

### Phosphate-Buffered Saline (PBS)

137 mM NaCl
2.6 mM KCl
10 mM $Na_2HPO_4$
1.8 mM $KH_2PO_4$
Adjust the pH to 7.4 with HCl

### Trypsin–EDTA

0.53 mM tetrasodium ethylenediamine-tetraacetic acid (EDTA)
0.05% trypsin

### Growth Medium

500 ml of Dulbecco's Modified Eagle Medium (DMEM) (high glucose, with L-glutamine, without sodium pyruvate)
5 ml of 200 mM L-glutamine
5 ml of penicillin (5000 U/ml)–streptomycin (5000 µg/ml) mixture
50 ml of fetal bovine serum, heat inactivated

## REFERENCE

1. Wyborski, D. L., and Vaillancourt, P. (1999) *Strategies* 12(1):1–4.

## MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.

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# Complete Control Cell Lines

Catalog #222100, #222105, and #222110

# QUICK-REFERENCE PROTOCOL

## Starting the Cells in Culture

- Transfer 10 ml of growth medium to a 15-ml conical tube
- Thaw the frozen cells quickly in a 37°C water bath
- Transfer the cell suspension to the conical tube containing the growth medium
- Centrifuge the cells at 200 × *g* for 5 minutes at room temperature and remove the growth medium by aspiration
- Add 10 ml of growth medium containing G418 to a 75-cm$^2$ tissue culture flask, resuspend the cells in the conical tube in 5 ml of growth medium, and transfer the cell suspension to the tissue culture flask
- Place the cells in a 37°C incubator with 5–7% $CO_2$

## Passaging the Cells

- Remove the growth medium, wash the cells with PBS, and remove the PBS by aspiration
- Add 1.5 ml of trypsin–EDTA to each 75-cm$^2$ tissue culture flask, incubate the cells at room temperature for ~2–5 minutes, and tap the flask to release the adherent cells
- Dilute the cells with the growth medium containing G418 and transfer the cells to the desired number of tissue culture flasks
- Place the cells in a 37°C incubator with 5–7% $CO_2$

## Freezing the Cells for Long-Term Storage

- Remove the growth medium, wash the cells with PBS, and remove the PBS by aspiration
- Add 1.5 ml of trypsin–EDTA to each 75-cm$^2$ tissue culture flask containing cells (2 ml/175-cm$^2$ flask), incubate the cells at room temperature for ~2–5 minutes, and tap the flask to release the adherent cells
- Dilute the cells with 8.5 ml of growth medium
- Transfer the cell suspension to a 15-ml conical tube, centrifuge at 200 × *g* for 5 minutes at room temperature, and remove the growth medium by aspiration
- Resuspend the cells in 1–2 ml of freezing medium
- Transfer the cells to cryovials, place the cryovials in a chilled controlled-freezing container, and incubate the cryovials at –80°C overnight
- The following day, transfer the cryovials to liquid nitrogen for long-term storage