Expresso™

Mammalian Cells – CHO-K1

Genlantis
A division of Gene Therapy Systems, Inc.

Catalog #	Description	Amount
C502100	Expresso™ Single Shot Mammalian Cells – CHO-K1	1 vial, 1 x 10^6 cells
C502200	Expresso™ Double Shot Mammalian Cells – CHO-K1	2 vials, 2 x 10^6 cells

Storage:	Store at -80ºC, in an area of the freezer where there is minimal temperature fluctuation. Cells can also be stored in the vapor phase of liquid nitrogen.

Related Products	Catalog #
Expresso™ Single Shot Mammalian Cells – HeLa S3, 1x10^6 Cells	C501100
Expresso™ Double Shot Mammalian Cells – HeLa S3, 2x10^6 Cells	C501200
Expresso™ Single Shot Mammalian Cells – HEK 293, 1x10^6 Cells	C503100
Expresso™ Double Shot Mammalian Cells – HEK 293, 2x10^6 Cells	C503200
Expresso™ Single Shot Mammalian Cells – NIH-3T3, 1x10^6 Cells	C504100
Expresso™ Double Shot Mammalian Cells – NIH-3T3, 2x10^6 Cells	C504200
Expresso™ Single Shot Mammalian Cells – COS-7, 1x10^6 Cells	C505100
Expresso™ Double Shot Mammalian Cells – COS-7, 2x10^6 Cells	C505200
TrojanPORTER™ Transfection Reagent, 375 reactions	T901007
TrojanPORTER™ Transfection Reagent, 750 reactions	T901015
TrojanPORTER™ Transfection Reagent, 3,750 reactions	T901075
TrojanPORTER™ Transfection Reagent, 7,500 reactions	T901150

INTRODUCTION

The Expresso™ Single-Shot and Double-Shot CHO-K1 Mammalian Cells are pre-counted, pre-aliquoted versions of their standard counterpart cell lines. However, they have been frozen in a state of suspended high efficiency. This means that all you need to do is thaw them, plate them, and transfect them 3 hours later. This time savings allows you to transfect at a moment's notice, without having to passage your cells for a day or longer prior to transfecting. Additionally, Expresso™ Cells were developed to significantly increase the protein expression yield for a given culture vessel surface area. Because Expresso Mammalian Cells can be plated at a much higher cell density (about 4X) than traditional passaged cells, up to 5 fold of protein yield enhancement can be achieved. This makes Expresso Mammalian Cells ideal for small scale or high-throughput testing of expression constructs, in which increased protein yield means enhanced assay sensitivity. Each lot of Expresso Mammalian Cells is functionally tested to ensure lot-to-lot consistency in terms of high cell viability and protein expression capability. The Expresso Mammalian Cells are not genetically modified and can be propagated and passaged like regular cells. Furthermore, their morphology is identical to passaged cells after 24 hours at 37ºC and 5% CO_2.

MATERIALS AND METHODS

Materials Required But Not Provided

1. Fetal bovine serum, USDA qualified (FBS)
2. DMEM Media
3. Non-Essential Amino Acids Solution, 100X (10 mM)=NAA
4. Micro-centrifuge or picofuge
5. Standard tissue culture-treated culture vessels/flasks/plates

Methods

IMPORTANT: Perform all steps in a Laminar Flow Hood to maintain sterility. All conditions given below have been optimized using the TrojanPORTER™ Transfection Reagent (see Related Items table above)

A. **Plating Expresso HeLa Mammalian Cells**

Transfection reagent manufacturers typically recommend a range of passaged cell plating densities based on the surface area of the culture vessel. We recommend seeding the Expresso CHO-K1 cells at 4X the transfection reagent manufacturer's highest recommended cell plating density three hours prior to transfection (see Step 3 below). Depending on the reagent and the demands of the experiment, optimization of DNA:transfection reagent ratio with a range of cell plating densities may be necessary.

1. Pre-warm DMEM medium and FBS in a 37ºC water bath for at least 15 minutes before use.
2. Add 10 ml of FBS and 1 ml of NAA to 90 ml of DMEM; this will make the CHO-K1 plating medium.
3. Determine the number of cells needed for your experiment. We recommend starting at a plating density that is 4X the Manufacturer's recommended passaged cell plating density but not exceeding the highest recommended cell plating density shown in **Table 1.**

Table 1: Highest Recommended Plating Densities for Expresso™ CHO-K1 Mammalian Cells

Cell Culture Plate	Well Diameter (mm)	Growth Area (cm^2)	Highest Recommended Plating Density	Total Volume Per Well (ml)
100 mm Dish	100	55	10.0 x 10E6	11-16.5
60 mm Dish	60	21	5.0 x 10E6	4.2-6.3
35 mm Dish	35	8	2.5 x 10E6	1.6-2.4
6 Well	34.8	9.5	1.5 x 10E6	2.0
12 Well	22.1	3.8	8.0 x 10E5	1.0
24 Well	15.6	1.9	4.0 x 10E5	0.50
48 Well	11	0.95	2.0 x 10E5	0.25
96 Well	6.4	0.32	1.0 x 10E5	0.10

Note: For robotic equipment, make the necessary allowances for dead volume.

IMPORTANT: **It is critical to follow steps 4-7 exactly achieve the highest cell viability.**

4. Rapidly thaw the cryovial(s) in a 37ºC water bath until a sliver of ice crystal remains.
5. Immediately centrifuge at 2,000g for 1 minute (a bench-top picofuge rated to spin at 2,000g can be used).
6. Remove supernatant using a P-1000 pipette to minimize disturbing the pellet (avoid aspirating with a vacuum line).
7. Use a P-1000 pipette to gently dislodge and break up the pellet in the cryovial using 1 ml of CHO-K1 plating medium.
8. Transfer the suspended cells to a sterile conical tube (the conical tube should be large enough to accommodate your final plating volume) and continue gently pipetting up and down to get a single-cell suspension.
9. Add CHO-K1 plating medium to the desired plating volume for your experiment. Mix gently by pipetting with a serological pipette and plate.
   OPTIONAL: Verify cell count and viability at this step using trypan blue staining and a hemocytometer (we recommend making a 1:4 dilution of cell suspension to trypan blue). Accurate cell counts can be achieved when > 200 cells are counted. Once you have verified the number of cells, dilute the cells by adding plating medium to the desired volume for your experiment. Mix gently by pipetting with a serological pipette and plate.
10. After plating, gently shake (not swirl) the plate to evenly distribute the cells. Place the plate into a 37ºC incubator with 5% CO_2 for three hours.
11. After incubating for 3 hours the cells will be adhered to the plate (>80%) and are ready for transfection. Do not exceed 4 hours, as transfection efficiency will begin to decline.
    IMPORTANT:
    a. Do not use antibiotics, as they may adversely affect the adherence and transfection efficiency of Expresso cells.
    b. Verify adherence and confluency under a light microscope before transfecting. The majority (>80%) of cells will be attached after 3 hours. The cells will appear round, with some beginning to spread out. Due to high-density plating, cell morphology will appear more compact when compared to regular passaged cells.
    c. Cells may be loosely attached to the plate surface. Use caution if you have to change or add media so as not to dislodge them.
    d. If plating for microscopy, we recommend using Poly-D-lysine coated glass bottom dishes (MatTek Corporation).
    e. It is very important to transfect the cells after approximately 3 hours in order to achieve maximum transfection efficiency.
    f. Remaining cells can be passaged in IMDM and used like normal passaged CHO-K1 cells.

B. **Transfection**

Follow your transfection reagent's recommended transfection protocol.

a. We recommend using CHO-K1 plating medium unless the transfection reagent requires serum-free media.
b. Transfect using the CHO-K1 plating medium.
c. If serum-free media is used, add 1 volume of the same serum-free media with 20% FBS to the transfected cells after the initial recommended incubation time without serum (usually 3-4 hours).
d. For maximum protein expression and yield, we recommend a minimal transfection time of 48-hours.
e. After 24-48 hours, examine the cells under a light microscope. The cells will appear crowded and compact; however there should be little or no cell death or debris in the media compared to regular passaged cells.

C. Cell Lysis

Aspirate the media and add the lysis buffer directly to the cells without washing. If washing is required, use care to avoid dislodging cells.

D. Subculture

For continuous subculture post-transfection, we recommend using CHO-K1 plating medium, i.e. DMEM supplemented with 10% (V:V) FBS and 1% (V:V) Non-Essential Amino Acids (10mM).

E. Troubleshooting

Low expression level and/or cell viability: titration of the DNA amount and the DNA:transfection reagent ratio relative to cell density may be required to optimize transfection efficiency and viability.

*US Patent Pending

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