CelliMax® TransCHO Kit02

Instructions for Use

Cat. No.: **TSCH02 (Liquid)**

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Product Description

CelliMax® TransCHO Kit02 consists of four components: CelliMax® CHO P02 Basal Medium, CelliMax® Uni FM03 Feed Medium, CelliMax® CHO Enhancer, and CelliMax® CHO Trans Reagent02. The kit is chemically defined (CD) and free of serum, animal-derived components, and hydrolysates. It supports highly efficient transient transfection and protein expression in various CHO cell lines cultured in high-density suspension.

Product Information

Name	Component	Glucose	Specification
CelliMax® TransCHO Kit02	CelliMax® CHO P02 Medium	6 g/L	1 L
	CelliMax® Uni FM03 Medium	80 g/L	200 mL
	CelliMax® CHO Enhancer	×	5 mL
	CelliMax® CHO Trans Reagent02	×	7 mL

Applications

CelliMax® TransCHO Kit02 is designed for transient expression in CHO cell lines, including CHO-K1, CHO-S, and CHO-DG44. The CelliMax® CHO P02 Medium can be used for cell thawing, passaging, transient expression, and cryopreservation.

Storage Conditions

Store at 2–8 ℃, protected from light.

Shelf Life

12 months.

Preparation Before Use

✧ CelliMax® Uni FM03 Medium contains a stabilized form of glutamine and does not require additional supplementation.

✧ Concentrated glucose solution (**stock concentration: 360–400 g/L**).
Note: Dissolve 360–400 g dextrose anhydrous in 1 L High Purity Water (such as Water for Injection or equivalent); sterile-filter prior to use.

Culture Guidelines

- Cell Culture

Table 1 Shaking incubator culture conditions

Parameter	Value
Orbital diameter	50 mm
Shaking speed	90–135 rpm (shake flask)
Temperature	37 ℃
CO_2 concentration	5–8%
Humidity	80 ± 5%

- Cell Thawing

a) Rapidly thaw a vial of frozen cells in a 37 ℃ water bath (< 2 minutes).

b) Aseptically transfer the cell suspension to a 50 mL centrifuge tube containing 15 mL pre-warmed CelliMax® CHO P02 Medium.

c) Centrifuge at 115 × g for 5 minutes.

d) Aspirate and discard the supernatant.

e) Resuspend the cell pellet in pre-warmed CelliMax® CHO P02 Medium and transfer to a 125 mL shake flask. The initial seeding density should be > 0.3 × 10^6 cells/mL.

f) Incubate in a shaking incubator under the conditions described in Table 1.

- Cell Passaging

a) Prewarm CelliMax® CHO P02 Medium for 20–30 minutes.

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b) Ensure a viable cell density ≥ 1.0 × 10^6 cells/mL and viability ≥ 90%, with cells maintained in the mid-exponential growth phase.

c) Based on the final culture volume, determine the appropriate volume of cell suspension to inoculate into a new shake flask, with a recommended initial seeding density of 0.3–0.6 × 10^6 cells/mL.

d) Aseptically transfer the calculated volume of cell suspension into a shake flask containing pre-warmed CelliMax® CHO P02 Medium.

e) Incubate in a shaking incubator under the conditions described in Table 1.

f) Passage cells every 2–4 days using fresh CelliMax® CHO P02 Medium to maintain optimal performance by following the above steps.

- Cell Adaptation

Direct Adaptation

1) CHO cells cultured in other serum-free media can be directly adapted to CelliMax® CHO P02 Medium. Refer to **Cell Passaging**.

2) The cells are considered adapted to CelliMax® CHO P02 Medium after at least two passages, with viable cell density > 2.0 × 10^6 cells/mL and viability > 90%.

Sequential Adaptation

1) If direct adaptation is not satisfactory, sequential adaptation may be used instead. The detailed sequential adaptation steps are shown in Table 2.

2) Passage the cells into a mixture of the original culture medium and CelliMax® CHO P02 Medium, and gradually increase the proportion of CelliMax® CHO P02 Medium. The detailed sequential adaptation steps are shown in Table 2.

3) The cells are considered adapted to CelliMax® CHO P02 Medium after at least two passages, with viable cell density > 2.0 × 10^6 cells/mL and viability > 90%.

- Cell Cryopreservation

a) Harvest cells in mid-exponential growth phase with viability > 90%.

b) Determine the viable cell density and calculate the volume of cryopreservation medium required to achieve a final cell density of ≥ 1.0 × 10^7 cells/mL.

c) Prepare cryopreservation medium consisting of 90% CelliMax® CHO P02 Medium and 10% dimethyl sulfoxide (DMSO). Store at 2−8 ℃ prior to use.

d) Centrifuge at 115 × g for 5 minutes, discard the supernatant, then resuspend the cell pellet in pre-equilibrated cryopreservation medium.

e) Promptly transfer the cell suspension into sterile cryovials.

f) Freeze using a controlled-rate freezing method (approximately -1 ℃/min) according to standard procedures.

g) Transfer frozen cells to liquid nitrogen (-196 ℃) for long-term storage.

Table 2 Sequential adaptation scheme

Ratio (Original: CelliMax® CHO P02 Medium) (%)	Seeding density (× 10^6 cells/mL)	Acceptance criterion to proceed to next adaptation step
100:0	0.3–0.6	≥ 2 passages with cell density > 2.0 × 10^6 cells/mL and viability > 95%
50:50	0.3–0.6	≥ 2 passages with cell density > 2.0 × 10^6 cells/mL and viability > 90%
0:100	0.3–0.6	≥ 2 passages with cell density > 2.0 × 10^6 cells/mL and viability > 90%

Transfection and Transient Expression

- Preparation of Cells for Transfection

Option 1: One day before transfection, adjust the cells to 3–5 × 10^6 cells/mL using fresh CelliMax® CHO P02 Medium. The seeding density may be adjusted based on the growth rate of the customer's cells, so that the cells reach 9–12 × 10^6 cells/mL with viability ≥ 95% on the day of transfection.

Option 2: On the day of transfection, centrifuge the cell suspension, discard the supernatant, and resuspend the cell pellet in fresh CelliMax® CHO P02 Medium to reach 9–12 × 10^6 cells/mL. Place the flask back in the shaking incubator and allow the cells to recover for 30 minutes.

Option 2 is recommended if further improvement in protein expression is desired.

- Cell Transfection

Table 3 Transfection protocol

Item	Recommended value
VCD	9–12 × 10^6 cells/mL
DNA	4 mg/L*
CelliMax® CHO Trans Reagent02	8 mL/L

The DNA concentration is recommended to be ≥ 800 ng/μL.

1) On the day of transfection (Day 0), add DNA at 4 mg/L directly to the cell suspension while gently swirling the flask.
2) Then add 8 mL/L CelliMax® CHO Trans Reagent02 directly to the cell suspension while gently swirling the flask.
3) Return the flask to the shaking incubator and continue incubation at 37 ℃ under the conditions specified in Table 1.

- Addition of Enhancer and Feed Medium

1) Add 0.3% CelliMax® CHO Enhancer (add only once), 6% CelliMax® Uni FM03 Medium, and an additional 6 g/L glucose 24 h post-transfection (Day 1) based on the initial culture volume, and switch the culture temperature from 37 ℃ to 32 ℃.
2) Add 6% CelliMax® Uni FM03 Medium and an additional 6 g/L glucose every 72 h (Day 4, 7, 10). The daily residual glucose level is recommended to be no less than 3 g/L during transient expression.
3) The harvest time can be flexibly adjusted based on predefined criteria (e.g., culture duration or viability < 60–70%), and protein expression levels should be tested.

Ordering Information

Table 4 Ordering information

Product	Order Reference No.	Specification	Format
CelliMax® TransCHO Kit02	TSCH02	1L Kit	Liquid
CelliMax® CHO P02 Medium	TSCH02-B01	1L	Liquid

For more information, please visit www.cellupro.com or contact sales@cellupro.com.

Taking a 30 mL transfection system in a 125 mL shake flask as an example

a) Add 120 μg DNA to the cell suspension while gently swirling the flask.
b) Add 240 μL CelliMax® CHO Trans Reagent02 to the cell suspension while gently swirling the flask.
c) Return the flask to the shaking incubator and continue incubation at 37 ℃.
d) Add 90 μL CelliMax® CHO Enhancer, 1.8 mL CelliMax® Uni FM03 Medium, and an additional 6 g/L glucose 24 h post-transfection (Day 1), and switch the culture temperature from 37 ℃ to 32 ℃.
e) Add 1.8 mL CelliMax® Uni FM03 Medium and an additional 6 g/L glucose every 72 h (Day 4, 7, 10). The daily residual glucose level is recommended to be no less than 3 g/L during transient expression.
f) The harvest time can be flexibly adjusted based on predefined criteria (e.g., culture duration or viability < 60–70%), and protein expression levels should be tested.

**Specifications are subject to change without prior notice, except for process changes.*

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