

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 °C, 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 °C
CO ₂ concentration	5–8%
Humidity	80 ± 5%

● Cell Thawing

- Rapidly thaw a vial of frozen cells in a 37 °C water bath (< 2 minutes).
- Aseptically transfer the cell suspension to a 50 mL centrifuge tube containing 15 mL pre-warmed CelliMax® CHO P02 Medium.
- Centrifuge at 115 × g for 5 minutes.
- Aspirate and discard the supernatant.
- 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 × 10⁶ cells/mL.
- Incubate in a shaking incubator under the conditions described in Table 1.

● Cell Passaging

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

- b) Ensure a viable cell density $\geq 1.0 \times 10^6$ cells/mL and viability $\geq 90\%$, 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 $0.3\text{--}0.6 \times 10^6$ 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 $> 2.0 \times 10^6$ 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 $> 2.0 \times 10^6$ 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 $\geq 1.0 \times 10^7$ cells/mL.
 - c) Prepare cryopreservation medium consisting of 90% CelliMax[®] CHO P02 Medium and 10% dimethyl sulfoxide (DMSO). Store at $2\text{--}8^\circ\text{C}$ prior to use.
 - d) Centrifuge at $115 \times g$ 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 $-1^\circ\text{C}/\text{min}$) according to standard procedures.
 - g) Transfer frozen cells to liquid nitrogen (-196°C) for long-term storage.

Table 2 Sequential adaptation scheme

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

Transfection and Transient Expression

● Preparation of Cells for Transfection

Option 1: One day before transfection, adjust the cells to $3\text{--}5 \times 10^6$ 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 $9\text{--}12 \times 10^6$ cells/mL with viability $\geq 95\%$ 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 $9\text{--}12 \times 10^6$ 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 °C 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 °C to 32 °C**.
- 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 °C.
- 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 °C to 32 °C.
- 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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