Trans CHO Kit Plus

User Guide

Product Introduction

Trans CHO Kit Plus is an upgraded transient protein expression system. The basal medium and feed medium are chemically defined and free of serum, hydrolysates, and animal-derived components. The product delivers high transfection efficiency and elevated protein expression, is compatible with multiple CHO cell lines, requires no incubation step, and supports automation workflows, thereby significantly improving operational efficiency.

Product Name	Catalog No.	Components	Intended Use	Form	Package Size
Trans CHO Kit Plus	33006	Trans Plus	Basal medium	Liquid	1 L
		ALLY Feed 100 Pro	Feed medium	Liquid	200 mL
		CHO Enhancer	Transfection enhancer	Liquid	5 mL
		Trans 1	Transfection reagent	Liquid	7.5 mL

Component Information

Component	Glutamine	Glucose	Growth Factors	Applicable Cell Lines
Trans Plus	With	8 g/L	Without	CHO-K1, CHO-S
ALLY Feed 100 Pro	With	150 g/L	Without	CHO-K1, CHO-S
CHO Enhancer	Without	Without	Without	CHO-K1, CHO-S
Trans 1	Without	Without	Without	CHO-K1, CHO-S

Storage and Stability

Component	Shelf Life	Storage Conditions
Trans Plus	12 months	Store at 2–8 °C away from light
ALLY Feed 100 Pro	12 months	Store at 2–8 °C away from light, recommended to use within 3 months after opening
CHO Enhancer	12 months	Store at 2–8 °C away from light
Trans 1	12 months	Store at -20 °C for 1 year; at 2–8 °C, stable for 2 months; can be freeze-thawed up to 5 times

If precipitation or turbidity occurs, discontinue use.

Protocol - Cell Passaging

1. Pre-warm the basal medium at 37.0 °C for 20 minutes prior to use;
2. Measure the viable cell density. Calculate the required seed culture volume for a final passage density of 0.2 × 10^6 cells/mL;
3. Aseptically transfer the calculated volume of seed cell suspension into a shake flask containing the pre-warmed basal medium at the required volume;
4. Place the shake flask in a CO_2 incubator shaker set to 37.0 °C, 120 rpm @ 50 mm (reduce the shaker speed for shake flasks with a larger bottom area), and the CO_2 concentration is 5%-8%;

5. Subculture the cells every 3 days using fresh medium following the steps above;
6. If cells are transferred from a different medium to this basal medium, proceed to the next step only when the cell doubling time is consistent with that in the original medium.

Protocol - Transfection (Simple Process)

1. **Day -1 (one day prior to transfection)**: Adjust the cell density to 2.0 × 10^6 cells/mL (very critical). The cell density will reach approximately 6 × 10^6 cells/mL on the day of transfection;
   Day 0 (day of transfection): Taking a 20 mL culture volume as an example (for other culture volumes, related reagents need to be scaled proportionally);
2. Collect cell culture suspension and measure the cell density. If the cell density exceeds 6 × 10^6 cells/mL, dilute the cells to 6 × 10^6 cells/mL with fresh medium. No adjustment is required when the cell density is between 5.5 × 10^6 and 6.5 × 10^6 cells/mL;
3. Add plasmid directly to the cell suspension (dropwise addition is not required): 1.5 μg/mL light chain and 1.5 μg/mL heavy chain (light:heavy ratio 1:1). If the heavy chain has been modified or is difficult to express, increase its proportion; if the light chain is difficult to express, increase its proportion. Minimum total plasmid concentration is 2.2 μg/mL. For a difficult-to-express molecule, total plasmid concentration may be increased to 4–5 μg/mL;
4. Add Trans 1 at 7.5 μL/mL (i.e., 150 μL Trans 1 for the 20 mL system) to the cell suspension. Mix rapidly immediately after addition to ensure uniform distribution of the mixture;

Plasmid Amount	2.2–3.0 μg/mL	3–4 μg/mL	4–6 μg/mL
Trans 1 Amount	7.5 μL/mL	8.0 μL/mL	8.5 μL/mL

5. Place the shake flask in a cell culture shaker at 37.0 °C after transfection, 120 rpm @ 50 mm (increase to 130 rpm for 25 mm orbit), and the CO_2 concentration is 5%;
6. **Feeding protocol for cells tolerant to high nutrient levels:**
   Day 1 (approximately 24 h post-transfection): Add 0.3% CHO Enhancer and 7% ALLY Feed 100 Pro relative to the transfection volume. Reduce temperature to 33 °C;
   Days 4–13: Add 7% ALLY Feed 100 Pro every 3 days.
   Note: *If temperature is not reduced or is reduced to > 33 °C, additionally add 2 g/L glucose.*
   For cultures lasting less than 7 days, add 0.3% CHO Enhancer and 9% ALLY Feed 100 Pro on Day 1, reduce temperature to 32 °C, and harvest on Day 7.
7. **Feeding protocol for cells not tolerant to high nutrient levels:**
   Day 1 (approximately 24 h post-transfection): Add 0.3% CHO Enhancer and 4% ALLY Feed 100 Pro relative to the transfection volume. Reduce temperature to 33 °C;
   Days 3–13: Add 4% ALLY Feed 100 Pro every 2 days.

Protocol - Transfection (High-Density Process)

1. **Day -1 (one day prior to transfection)**: Adjust the cell density to 3.0 × 10^6 cells/mL (very critical). The cell density will reach approximately 9 × 10^6 cells/mL on the day of transfection;
   Day 0 (day of transfection): Taking a 20 mL culture volume as an example (for other culture volumes, related reagents need to be scaled proportionally);
2. Measure cell density and adjust to 8–11 × 10^6 cells/mL using fresh medium (centrifugation-based medium exchange is the preferred method);
3. Add plasmid directly to the cell suspension (dropwise addition is not required): 1.5 μg/mL light chain and 1.5 μg/mL heavy chain (light:heavy ratio 1:1). If the heavy chain has been modified or is difficult to express, increase its proportion; if the light chain is difficult to express, increase its proportion. Minimum total plasmid concentration is 2.2 μg/mL. For a difficult-to-express molecule, total plasmid concentration may be increased to 4–5 μg/mL;
4. Add Trans 1 at 7.5 μL/mL (i.e., 150 μL Trans 1 for the 20 mL system) to the cell suspension. Mix rapidly immediately after addition to ensure uniform distribution of the mixture;

Plasmid Amount	2.2–3.0 μg/mL	3–4 μg/mL	4–6 μg/mL
Trans 1 Amount	7.5 μL/mL	8.0 μL/mL	8.5 μL/mL

5. Place the shake flask in a cell culture shaker at 37.0 °C after transfection, 120 rpm @ 50 mm (increase to 130 rpm for 25 mm orbit), and the CO_2 concentration is 5%;
6. **Feeding protocol for cells tolerant to high nutrient levels:**
   Day 1 (approximately 24 h post-transfection): Add 0.3% CHO Enhancer and 7% ALLY Feed 100 Pro relative to the transfection volume. Reduce temperature to 33 °C;
   Days 4–13: Add 7% ALLY Feed 100 Pro every 3 days.
   Note: If temperature is not reduced or is reduced to > 33 °C, additionally add 2 g/L glucose;
   For cultures lasting less than 7 days, add 0.3% CHO Enhancer and 9% ALLY Feed 100 Pro on Day 1, reduce temperature to 32 °C, and harvest on Day 7.
7. **Feeding protocol for cells not tolerant to high nutrient levels:**
   Day 1 (approximately 24 h post-transfection): Add 0.3% CHO Enhancer and 4% ALLY Feed 100 Pro relative to the transfection volume. Reduce temperature to 33 °C;
   Days 3–13: Add 4% ALLY Feed 100 Pro every 2 days.

Protocol - Cell Cryopreservation

1. Prepare cells in the log phase with viability > 95%;
2. Measure viable cell density. Calculate the required volume of cryopreservation medium to achieve a final density of 1.0–1.5 × 10^7 cells/mL;
3. Prepare cryopreservation medium: 90% Basal Medium + 10% DMSO. Store at 2–8 °C;
4. Centrifuge at 200 × g for 5 minutes. Resuspend the cell pellet in cryopreservation medium to the desired final volume;
5. Aliquot the cell suspension into appropriate cryovials;
6. Place the cryovials in a controlled-rate freezing container and follow the standard freezing protocol;
7. Transfer the cryovials to liquid nitrogen for long-term storage.

Protocol - Cell Recovery

1. Pre-heat a water bath to 37.0 °C;
2. Remove the cryovial from liquid nitrogen and quickly thaw the frozen cells in the water bath (less than 3 minutes);
3. Transfer the entire cell suspension from the cryovial into a 125 mL shake flask containing 30 mL of pre-warmed basal medium;
4. Place the flask in a shaker at 37.0 °C, 5%–8% CO_2, 120 rpm @ 50 mm;
5. Passage the cells at least twice. Once cells are fully recovered with viability above 90%, proceed with planned experiments.

Special Note

1. If basal medium has been stored for more than 6 months from the date of manufacture, supplement with 2–4 mM glutamine (Gln) prior to use.
2. Store Trans 1 transfection reagent at -20 °C if not used within 3 months.