Trans 293 Kit Pro

User Guide

Product Introduction

The Trans 293 Kit Pro includes the basal medium CD 293 01 and the feed medium ALLY 293 Feed. Both are chemically defined, serum-free, hydrolysate-free, and free of any animal-derived components. This product offers high transfection efficiency and high expression yields, making it suitable for high-density suspension culture of various HEK293 cell lines.

Product Name	Catalog No.	Component	Intended Use	Form	Package Size
Trans 293 Kit Pro	33007	CD 293 01	Basal medium	Liquid	1 L
		ALLY 293 Feed	Feed medium	Liquid	100 mL
		293 PEI Plus	PEI transfection reagent	Liquid	5 mL

Component Information

Component	Glutamine	Glucose	Growth Factors	Applicable Cell Lines
CD 293 01	With	6 g/L	Without	HEK293F, Expi293F, 293T
ALLY 293 Feed	Without	120 g/L	Without	HEK293F, Expi293F, 293T
293 PEI Plus	Without	Without	Without	HEK293F, Expi293F, 293T

Storage and Stability

Component	Shelf Life	Storage Conditions
CD 293 01	12 months	Store at 2–8 °C away from light
ALLY 293 Feed	12 months	Store at 2–8 °C away from light
293 PEI Plus	12 months	Store at 2–8 °C away from light

If precipitation or turbidity occurs, discontinue use.

Protocol - Cell Passaging

1. Pre-warm basal medium at 37.0 °C for 20 minutes prior to use;
2. Measure the viable cell density. Calculate the required seed culture volume based on a final seeding density of 0.4 × 10^6 cells/mL;
3. Aseptically transfer the calculated volume of seed cell suspension into a shake flask containing the pre-warmed basal medium of 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 basal medium following the same procedure above;
6. If cells are transferred from another medium to basal medium, proceed to the next step only when the cell doubling time is consistent with that in the original medium;

Protocol - Transfection

1. **Day -1 (one day prior to transfection)**: Adjust the cell density to 2.0 × 10^6 cells/mL (this step improves process stability and expression yield). The cell density will reach approximately 4 × 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 4.5 × 10^6 cells/mL, dilute the cells to 4.0 × 10^6 cells/mL with fresh medium (centrifugation-based medium exchange is the preferred method, as it can increase expression yield by 50%);
3. Dilute 30 μg of plasmid (final concentration is 1.5 μg/mL) in 10% of the culture volume using CD 293 01 medium. Then add 104 μL of 293 PEI Plus (final concentration is 5.2 μL/mL, PEI:DNA = 3.5:1), mix thoroughly by pipetting, and let stand for 5–20 minutes;
4. Slowly add the mixture dropwise while mixing to ensure even distribution;

Single Plasmid	Dual Plasmid	Other Cases
Total DNA: 1.5 μg/mL	Light chain: 0.8 μg/mL Heavy chain: 0.7 μg/mL	Total light chain: 0.8 μg/mL Total heavy chain: 0.7 μg/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. **Day 1 (approximately 24 hours post-transfection)**: Add 8% ALLY 293 Feed (based on the culture volume). Temperature shift is not required. Harvest between **Day 5 and Day 7** (any day within this range; longer culture yields higher expression).

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

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.