Neo T-HEK Transfection Kit 1 x Transfection Enhancer T-HEK-E-2.5ML 1 x Transfection Booster T-HEK-B-100ML

#Cat: NB-58-0079 Size: Kit

General Information

The Neo T-HEK Transfection Reagents provide the ultimate solution for achieving maximal transfection efficiency using PEI (polyethylenimine) prior to viral vector or recombinant protein production. Both reagents are chemically defined and serum free, specifically designed to deliver exceptional transfection results in animal component-free cultivation systems. Neo T-HEK Transfection Enhancer promotes the process of the DNA:PEI complex formation and is supplemented directly in this transfection step. Neo T-HEK Transfection Booster has been uniquely designed to boost efficiency of co-transfection of multiple plasmids DNA into HEK293 cells and deliver superior transfection efficiency. Combined with Neo T-HEK T Medium (NB-58-0078), which can be used during stock culture, transfection, and production stages, the reagents offer a streamlined approach to process optimization

Product Specifications

Appearance	Clear light-yellow solution
Specifications	- Chemically defined - Serum-free - Animal derived component-free
Storage and Shelf Life	+2°C to +8°C; protected from light. Please refer to the label for expiry date.
Shipping Conditions	Ambient

Instructions for Use

Culture Conditions

Temperature	36.5°C
CO_2	7 %
Culture vessel	Shake flask
Shaking rate	155 rpm
Inoculation cell concentration	8×10^5 viable cells/ml

General Transfection Guidelines:

• Allow freshly thawed cells to recover in culture for three or more passages post-thaw and before transfection.

• During all steps, mix the cells by gentle swirling; avoid vigorous mixing/pipetting. Cell health is critical to maximal performance.

• Complexation of plasmid DNA and Transfection Reagent takes place at room temperature.

Protocol for Transfection

See Table 1 below for transfection at various scales. Subculture and expand cells every three days with 8×10^5 cells/ml inoculation cell concentration until cells reach a density of approximately $35 – 45 \times 10^5$ cells/ml.

Day 0: Cell Passaging

1. On the day the cells are split, determine the cell density and relative viability. The cell density should be around $35 – 45 \times 10^5$ cells/ml, with a viability of at least 85 %.
2. Seed the cells into fresh, pre-warmed Neo T-HEK T Medium, supplemented with 0.4 g/L L-Glutamine, to a final concentration of 8×10^5 cells/ml. Note: The inoculated flask will be used for transfection, so prepare a separate culture flask for stock cultures.
3. Incubate the cells in a 36.5°C incubator with 7 % CO^2 in a humidified environment on a shaker

Day 2: Transfection of Cells

4. Two days later, recheck the cell density and viability percentage. The cells should have a density of around $35 – 45 \times 10^5$ cells/ml with a viability of at least 85 % to proceed with the transfection.
5. Dilute the cells with fresh, pre-warmed Neo T-HEK T Medium, supplemented with 0.4 g/L L-Glutamine to a final density of 30×10^5 cells/ml.
6. Keep the cells in a 36.5°C incubator with 7 % CO_2on a shaker while preparing the DNA/transfection complex.
7. Prepare two separate tubes: Tube 1 for the PEI Reagent and Tube 2 for the plasmid DNA dilution. Note: Neo T-HEK T Medium should be used for both dilutions.
8. Dilute your PEI transfection reagent (as described in the instruction manual of the PEI supplier) and your plasmid DNA (to a final concentration of 1.5 µg/ml culture volume to transfect) with Neo T-HEK T Medium. Incubate for 10 minutes at room temperature.
9. Slowly add the contents of Tube 1 into Tube 2 drop by drop. Immediately add Neo T-HEK Transfection Enhancer with 2.25 µl/ml, gently mix the solution with a pipette three times, and allow it to incubate for exactly 8 minutes at room temperature to form the complex.
10. Gently mix the transfection complex twice with a pipette, then add it to the shake flask from step 5.
11. After two hours, add Neo T-HEK Transfection Booster with 62.5 µl/ml of transfection volume and incubate the cells for three days with shaking.
12. Measure transfection efficiency after 48 h or continue cultivation until harvest of the product.

Table 1: Recommended reagent volumes for transfection at various scales

Shake flask total volume	**250 ml**	**500 ml**	**1 L**
Culture volume to transfect	50 ml	100 ml	200 ml
Total Number of cells required	1.5×10^8	3×10^8	6×10^8
Total amount of plasmid DNA	75 µg	150 µg	300 µg
Neo T-HEK Transfection Enhancer	112.5 µl	225 µl	450 µl
Neo T-HEK Transfection Booster	3.125 ml	6.250 ml	12.5 ml

Precautions and Disclaimer

This product is for research use and further manufacturing only

Help Needed?

If you have any further questions regarding this product, please do not hesitate to contact our cell culture experts by email (info@neo-biotech.com) or phone (+33 9 77 40 09 09).