

293 SFM Kit Instructions for Transient Transfection and Protein Expression in Cells



【Product information】：

Product Name	Product Code	Package Specification	Storage Conditions	Shelf Life
ACE293 SFM	ACE04101	1000ml	Store protected from light at 2-8° C.	9months
ACE Trans	ACE05102	1ml*5	-20℃	12months
ACE ProEnhance	ACE05103	1ml*6	-20℃	12months
ACE PepPlus	ACE05104	20ml	Store protected from light at 2-8° C.	9months

Usage Advisory:

- (1) UV exposure prohibited - protect from all ultraviolet light sources;
- (2) Pre-warming unnecessary - use directly at 2-8°C;
- (3) Medium storage - maintain at 2-8°C in medical-grade refrigerator (do not freeze);
- (4) Reagent kit components:
 - Cryogenic reagents (-20°C storage): aliquot before freezing to avoid freeze-thaw cycles
 - Post-opening: Store at 4°C and use within 7 days

【Scope of Application】： For research use only. Not suitable for clinical diagnosis or treatment

【Main Ingredient】： The product is a serum-free culture medium primarily composed of carbohydrates, amino acids, inorganic salts, and trace elements.

【Intended Use】： This system is suitable for medium-to-high density suspension culture, transient DNA transfection, and protein expression in HEK293 and other 293 cell lines.

【Transport Requirements】：

ACE 293 SFM: Ship on wet ice (0-4° C) with light protection.

ACE ProEnhance: Ship on wet ice (0-4° C) with light protection.

ACE Trans: Ship on wet ice (0-4° C) with light protection.

ACE PepPlus: Ship on wet ice (0-4° C) with light protection.

1.Experimental Procedure

This experimental method was developed based on the suspension culture experience of HEK293 cells in Erlenmeyer flasks, but this protein expression system is also applicable to DNA transfection and recombinant protein expression in other suspension-cultured 293 cells.

1.1 Pre-transfection Cell Culture

- (1) Culture HEK293 cells in a 5% CO₂ constant-temperature shaker at 37° C and 120 rpm. During subculture, perform cell counting and viability assessment first, and preferably select cells with high viability at a density of $3-5 \times 10^6$ cells/ml for subculture. Cells cultured at excessively high densities may exhibit changes in growth status after subculture, such as slowed growth rate or decreased culture density, which can directly affect subsequent applications like recombinant protein expression.
- (2) For cell subculture, the recommended post-subculture cell density is 0.6×10^6 cells/ml, with subculturing performed every 2-3 days. This culture medium can support a maximum cell density of approximately 1.3×10^7 cells/ml, with cell viability generally remaining above 95% even at this density.
- (3) If excessive dead cells are observed during culture, discard the cells and use fresh cells.

Key Operating Points and Precautions:

Recommended shaker parameters: Temperature 36-37° C, CO₂ concentration 5%, rotation speed ≤ 120 rpm (amplitude 26). The calculation formula for shakers with different amplitudes is as follows:

or amplitude 50, calculate according to the formula: $m \times \omega_1^2 \times \frac{26}{2} = m \times \omega_2^2 \times \frac{50}{2}$, therefore the relationship between ω_1 and ω_2 is: $\omega_1 \approx 1.4\omega_2$, which is approximately 86 rpm.

1.2 Preparation of Cells for Transfection

- (1) Prior to transfection, determine the cell density and viability. To ensure optimal transfection efficiency, it is recommended to use cells in the logarithmic growth phase (density approximately $2-5 \times 10^6$ cells/ml) with viability >98%;
- (2) Centrifugation of cells is not required. If the cell density is 2.0×10^6 cells/ml, proceed directly with transfection. If the cell density exceeds 2.0×10^6 cells/ml, dilute with fresh ACE293 SFM medium to adjust the density to 2×10^6 cells/ml (users may also optimize the transfection density based on their experience);
- (3) Place the shake flask in a 5% CO₂ constant-temperature shaker and incubate at 37° C with constant agitation at 120 rpm for 10 minutes before initiating transfection.

Key Operating Points and Precautions:

- a. Ensure cells are in logarithmic growth phase ($2-5 \times 10^6$ cells/ml). When cell density exceeds 2×10^6 cells/ml, resuspend in fresh culture medium. Using 293 cells not in logarithmic phase will result in reduced transfection efficiency and expression levels;
- b. For clients not using cell counters: Due to potential counting inaccuracies, maintain transfection density at $1.8-2.5 \times 10^6$ cells/ml;
- c. Maintain cell viability above 98%. Suboptimal cell conditions will adversely affect transfection efficiency, expression levels, and duration of expression;
- d. After diluting cells to 2.0×10^6 cells/ml, equilibrate cell suspension in shaker for 10 minutes before transfection. Transfection efficiency correlates with plasmid size. For larger plasmids, recommend transfection at lower cell densities. Clients should optimize cell density ($\leq 2.5 \times 10^6$ cells/ml) based on their specific plasmid size.

1.3 Transient Transfection (using 100ml cell suspension as example)

- (1) Prepare two 15ml sterile centrifuge tubes. To one tube, add 5ml ACE293 SFM and 100 μ g sterile plasmid DNA, mix gently by pipetting; take the other tube, add 5ml ACE293 SFM and 500 μ l ACE Trans transfection reagent, mix gently by pipetting (see Figure 1);
- (2) Transfer all liquid from the tube containing transfection reagent to the tube containing plasmid, mix gently by pipetting;
- (3) Let stand at room temperature for 10min to complete preparation of plasmid-vector complexes;
- (4) Remove cells from constant-temperature shaker, slowly add the prepared plasmid-vector complexes while shaking, then return to CO₂ constant-temperature shaker for suspension culture. After 3 hours, appropriate antibiotics may be added if needed.

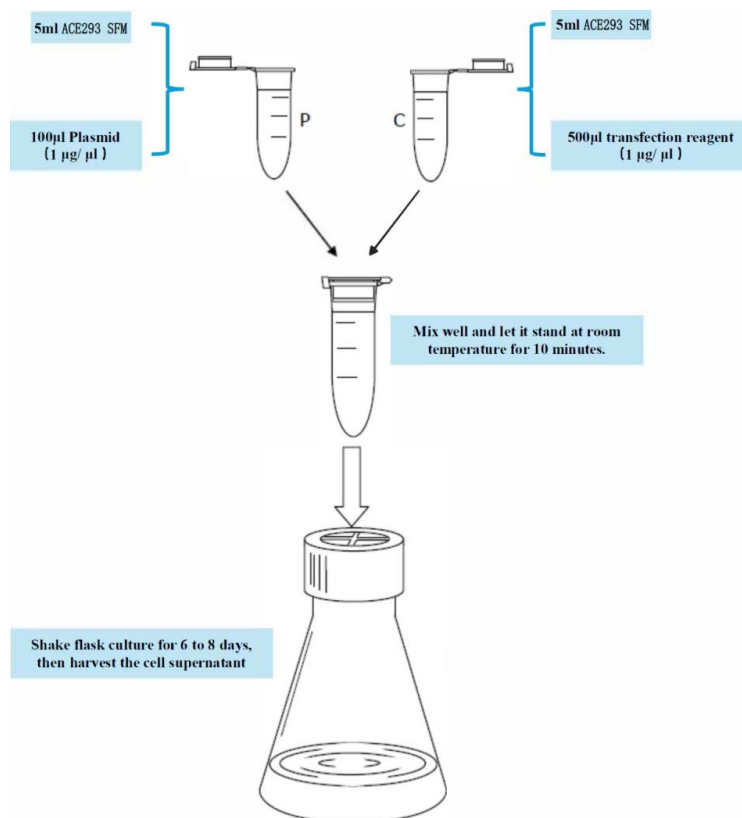


Figure 1. Preparation of plasmid-vector complex

Operation Points and Precautions:

- a. When preparing plasmid-vector complexes, the total amount of ACE293 SFM used should be 1/10 of the cell suspension volume; plasmid DNA dosage is 1 μ g/ml (DNA/total volume of cell culture medium for protein production);
- b. The mass ratio of plasmid DNA to transfection reagent can be 1:3, 1:4, 1:5, or 1:6. For most plasmids, a ratio of 1:5 works best. Users may optimize conditions based on their experience, but ratios below 1:3 or above 1:8 are not recommended;
- c. ACE Trans (concentration 1 μ g/ μ l) appears as a clear solution - mix well by shaking or pipetting before use;
- d. Do not shake during plasmid-transfection reagent incubation (vigorous agitation may cause incubation failure); Complete preparation of plasmid-vector complexes before removing cells from the shaker; Add complexes to cells dropwise while shaking - do not remove cells from the shaker too early.

1.4 Product Expression and Detection

- (1) At 24 hours post-transfection, 600 μ l of protein expression enhancer (ACEProEnhance) may be added to increase product yield;
- (2) The protein expression nutrient supplement (ACE PepPlus) should be added once at a 1:50 ratio 24 hours post-transfection;
- (3) Measure product expression levels between days 5-7 post-transfection. For unstable recombinant proteins (e.g., certain antigenic proteins), daily expression monitoring may begin from day 2 post-transfection to determine optimal harvest time based on protein stability;
- (4) For most recombinant proteins, peak expression levels are typically achieved around day 6 post-transfection. Clients may determine the optimal harvest time based on cell status and expression levels.

Operation Points and Precautions:

- a. ACEProEnhance stock concentration is 100%, working concentration is 0.6%. Cell growth rate may slow down after addition - this is normal;
 - b. ACE PepPlus is a plant hydrolysate-containing nutritional supplement that can be optionally added to replenish nutrients consumed in the medium, thereby increasing protein expression;
- When harvesting supernatant based on cell viability, it is recommended not to harvest when cell viability is below 70%.

2.Common Issues and Solutions

2.1 Low Transfection Efficiency

Through repeated experimental verification, for HEK293 cells in normal growth condition, this transfection system typically achieves 60%~80% transfection efficiency for fluorescent protein expression plasmids at 48 hours. Possible causes for abnormally low efficiency include:

- (1) Cell viability below 98%;
- (2) Slow cell growth rate;
- (3) Cell clumping before transfection (normally growing cells in ACE293 SFM generally don't clump - clumping may indicate contamination or excessive dead cells/secretion);
- (4) Plasmid DNA contaminated with proteins/other substances or high endotoxin levels;
- (5) Improper plasmid-to-transfection reagent ratio (optimal ratio varies by plasmid, typically 1:3 to 1:7);
- (6) Incorrect ACE293 SFM volume (add 10ml per 100ml cell suspension, i.e., 10:1 ratio).

2.2 Reduced Post-transfection Viability

Some viability reduction is normal (typically >80% at day 6). Users may extend culture duration to increase protein expression, but maintain viability above 70%.

2.3 Post-transfection Cell Clumping

Loose clumps may result from adherent cells detaching during shaking, often caused by improperly cleaned flasks or low viability.