ACE 293 SFM Instruction Manual



【Product Name】：

Product Name	Product Code	Package Specification	Storage Conditions	Shelf Life
ACE 293 SFM	ACE04101	1000ML	9months	Store at 2-8℃
ACE 293 SFM Basic	ACE04101-P	10L	24months	Store at 2-8℃

【Main Ingredient】： This product is a serum-free, chemically defined medium primarily composed of glucose, L-alanyl-L-glutamine (Ala-Gln), amino acids, inorganic salts and trace elements.

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

【Intended Use】: This product is a chemically defined, serum-free cell culture medium specifically formulated for medium-to-high density suspension culture and recombinant protein expression in HEK293 and other 293 cell lines.

【Transport Requirements】： Transport on wet ice

【Usage Method】

Important Usage Notes:

Avoid UV exposure – Do not irradiate the product with ultraviolet light.

No pre-warming required – Use directly without preheating.

Proper storage – Refrigerate the medium in a medical-grade refrigerator (do not freeze).

1.Liquid Medium Preparation Method (1000ml)

(1) 1. Weigh 20.73g of medium powder and 2.04g $NaHCO_3$ (for 5% CO_2 culture) separately; 2. Add to approximately 800ml of purified water and stir to dissolve for about 1 hour;

(2) Adjust pH to 7.0 using sodium hydroxide or hydrochloric acid. At this stage, the solution should show no visible powder particles (though slight turbidity may remain). Bring to final volume of 1000ml with purified water;

(3) Perform sterile filtration using 0.1 μm or 0.22 μm membrane filters. The filtered medium should appear completely clear and transparent;

(4) Store at 4° C;

(5) For suspension culture of 293 cells, supplement with F-68 (prepare by dissolving 10g F-68 powder in 80ml purified water with stirring until complete dissolution, then bring to 100ml with purified water. After

autoclaving, add to culture medium at 1:100 ratio for a final concentration of 1g/L).

2.Cell Medium Change and Subculture

(1) For newly received ambient-temperature cells, immediately transfer them to an appropriate sterile shake flask (the culture volume should be controlled within one-fifth of the flask's rated capacity), and incubate with shaking. When cell density recovers to 3-6×10^6 cells/ml, subculture or cell cryopreservation can be performed;

(2) For cryopreserved cells retrieved from liquid nitrogen tanks or cells transported on dry ice, please follow the cell recovery and culture methods described in section (2. Cell Cryopreservation and Recovery) below;

(3) During subculture, perform cell counting first. After confirming the density, centrifugation is not required - the cell suspension can be directly diluted into fresh medium at the desired ratio. The post-subculture cell density should be maintained at approximately 0.3×10^6 cells/ml (typically requiring subculture every 4 days), or at 0.6×10^6 cells/ml (generally requiring subculture every 3 days). This medium can support a maximum cell density of about 13×10^6 cells/ml, with cell viability typically remaining above 95% even at this density;

(4) If excessive dead cells are observed during shake culture, seed at a low cell density (below 5×10^5 cells/ml) for static culture until normal viability is restored, then resume shake culture.

3.Cell Cryopreservation and Recovery

3.1 Cryopreservation

(1) Prepare 7.5% dimethyl sulfoxide (DMSO) cryopreservation medium using cell culture medium, or use Zhuhai Kairy's ready-to-use serum-free cell cryopreservation medium KD-Freeze (Catalog No. K60001);
(2) Culture cells to logarithmic growth phase (density ~6×10^6 cells/ml), count and collect cells by centrifugation;
(3) Resuspend pelleted cells in cryopreservation medium at 5-20×10^6 cells/ml;
(4) Aliquot cell suspension into labeled cryovials, ensuring tight sealing of caps;
(5) Place cryovials in -80° C freezer for gradual cooling;
(6) Transfer cryovials to liquid nitrogen for long-term storage the next day. Complete this transfer process as quickly as possible (recommended within 2 minutes). If cryovial temperature rises above -50° C during this process, cells may be rapidly damaged.

3.2 Recovery

(1) Retrieve cryovials from liquid nitrogen tank, dry ice or ultra-low temperature freezer, and immediately place in 37° C water bath until complete thawing;
(2) After thawing, thoroughly wipe cryovial with 75% ethanol, then transfer entire cell suspension to 50ml sterile centrifuge tube using pipette. Slowly add culture medium to final volume of 20ml;
(3) Centrifuge at 1000rpm for 5min, discard supernatant containing cryoprotectant. When using Zhuhai Kairy's serum-free cryopreservation medium, directly transfer recovered cells to shake flask containing AC 293 SFM medium for resuspension;
(4) Resuspend cells in fresh medium at 0.6-1.0×10^6 cells/ml, then inoculate in shake flask for suspension culture;
(5) After 3-6 days culture, count cells to monitor density and viability recovery. When density reaches 3-6×10^6 cells/ml, perform subculture. Normal growth status typically resumes after 1-2 passages;

(6) If post-thaw cell density is too low, first perform static culture in flask until density recovers to 0.6-1.0×10^6 cells/ml, then transfer to shaker for suspension culture.。

Key Operating Points and Precautions:

Recommended incubator 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:

For an amplitude of 50, the calculation follows the formula:$m\times\omega 12\times\frac{26}{2}=m\times\omega 22\times\frac{50}{2}$，thus the relationship between ω_1 and ω_2 is: $\omega 1\approx 1.4\,\omega 2$，corresponding to approximately 86 rpm.

2.If using other brands of culture media, it is recommended to gradually transition to ACE 293 SFM. Allow cells to undergo continuous passage for several weeks while monitoring cell viability and expansion rate. Proceed with subsequent experiments only after cells have returned to normal growth status.

Decreased cell density and viability

(1) When cell viability is 80%-95%:

Centrifuge cells to remove supernatant, resuspend in fresh culture medium to achieve a density of 3×10^6 cells/ml, and inoculate in shake flasks for oscillating culture. Monitor cell proliferation daily. If proliferation is observed, continue culturing until cell density reaches 9×10^6 cells/ml before subculturing. Maintain subculture density at 3×10^6 cells/ml until cell viability recovers above 95%, then restore subculture density to 0.3-0.6×10^6 cells/ml.

(2) When cell viability is 60%-80%:

Centrifuge cells to remove supernatant, resuspend in fresh ACE 293 SFM to achieve a density of 0.5×10^6 cells/ml, and inoculate in culture flasks for static culture. Observe cell growth every two days. If growth is observed, continue culturing until cell density reaches 2.0×10^6 cells/ml, then subculture at 0.5×10^6 cells/ml in culture flasks for static culture. Repeat this process until cell viability exceeds 90%, at which point cells can be transferred to shake flasks for culture with an inoculation density no less than 2.0×10^6 cells/ml. If adherent cells are observed during static culture, continue culturing. When cell density reaches a certain level, some cells will naturally detach, while remaining adherent cells can be dislodged by gentle pipetting.

(3) When cell viability is below 60%:

Discard the cells and obtain new cells.

(4)Cell clumping:

Check whether additional substances have been added to the culture medium. If using reusable glass shake flasks, ensure the bottles are thoroughly cleaned. If cell clumps appear spherical, this may be due to the addition of foreign substances or detergents. If cell clumps appear as flakes or irregular loose structures, this may be caused by residual contaminants adhering to the flask from previous cultures that were not properly cleaned, or due to low cell viability.