CESCO BIOENGINEERING
We Provide Cutting-Edge Technologies for Cell Engineering

Plus MDCK SFM, Powder

with L-glutamine
without sodium bicarbonate

Product Information Sheet

Introduction
Plus MDCK SFM is a component-defined cell culture medium, formulated without any human or animal-derived components. Plus MDCK SFM is designed to support the serum-free growth of MDCK cell line of interest in the areas of virology, virus production, and biotechnology.

Caution: Not intended for human or animal diagnostic or therapeutic uses.

Storage Conditions: 2 to 8°C, in the dark

Shelf Life: 1 year

Intended Use
Plus MDCK SFM is a component-defined, animal component-free and ultra-low protein medium, designed specifically for the growth of MDCK cell line. Plus MDCK SFM is particularly suitable for growing viruses.

Features of Plus MDCK SFM
- Very low protein concentration ~ 5 µg/mL
- No proteins or peptides of animal or human origin
- No complexes such as plant hydrolysate, yeast extract. Ease of downstream product purification
- Reduced risk of viral contamination
- Lot-to-lot consistency
- Equivalent cell growth and virus titers vs. serum-supplemented media

Preparing liquid medium
1.Measure out 5% less distilled water than desired final volume.
2.Add powder to 15 to 30 C (room temperature) D.I. water or equivalent grade water with gentle stirring.
3.Stir until dissolved. Please avoid extensive mixing with mixing time longer than 5 mins.
4.Add 3.0g of $NaHCO_3$ per liter of medium.
5.Stir until dissolved.
6.Adjust pH of medium to 0.2-0.3 below desired final working pH: use of 1N HCL is recommended.
7.Dilute to a desired volume with D.I. water.
8.Steritize immediately by membrane filtration. PVDF, or PES membrane is highly recommended.
9.Please store the culture medium in glass vessel, or plastic bag with LDPE contact layer. Plastic bag with EVA contact layer is not recommended.

Cell Culture Procedures

Adaptation of Cultures to Plus MDCK SFM
For MDCK cells apply to virus production or other applications, if original cells are cultured in serum-contained culture medium, no adaptation is needed. If original cells are already adapted in other serum-free culture medium, or the directly culture in Plus MDCK does not result a good cell growth, a weaning adaptation procedure might be required by mixing 50% original culture medium with Plus MDCK and culture until sub-confluence. Around 3 to 4 passages might be required for the cells adapted to Plus MDCK SFM.

Static Culture
Due to ultra-low protein concentration in Plus MDCK SFM, care must be taken to protect cells from being damaged by enzyme when making cell passage. We have found that modification on protocols may be required to obtain optimum results with Plus MDCK SFM. The following protocols have been used successfully in our laboratories.

1. Grow MDCK cells in tissue culture flasks (T-75) until 70~90% confluency.
2. Remove media from tissue culture flask (We recommend products from Corning Costar). Rinse flasks with 12 ml Dulbecco's PBS without Ca^{2+} or Mg^{2+} and remove.
3. Add 3 mL pre-warmed 0.05% Trypsin-EDTA to flask and dispense evenly over the entire surface area. Trypsin-EDTA is allowed to sit for 45-60 seconds at room temperature before being removed entirely.
4. Cultures are then incubated at 37℃ until cells become rounded (about 3 to 7 minutes depends on the confluency level) and are easily dislodged from the surface by shaking the flask. Please avoid rapping the flask. This might injure cells especially in serum-free culture environment.
5. Once detached, add 12 mL Plus MDCK SFM medium containing 500 µg/mL Soybean Trypsin Inhibitor (optional) to flask and transfer cell suspension to a 15 mL centrifuge tube.
6. Centrifuge for 5 minutes at 160 xg.
7. Remove supernatant and re-suspend cell pellet in 10 mL Plus MDCK SFM medium.
8. Determine viable and total cell number. Determine cell viability by trypan blue dye exclusion method, and use a hemocytometer to determine cell number
9. Seed flasks at 0.5-1.0x10^4 viable cells/cm^2.
10. Incubate at 37℃ in a humidified chamber containing of 5% CO2 until cells are 80-90% confluent, which takes 3 to 4 days after seeding. Due to the medium contains more buffers for virus production, higher CO2 concentration, e.g. 7% maybe required for the first day after inoculation if the culture medium tends to be with higher pH initially.

Roller Bottle culture

Pre-warm 0.2-0.25 ml of medium per cm^2 of roller bottle surface area at 37℃. Inoculate the roller bottle with about 0.5-1.0x10^4 cells/cm^2 to get an adequate distribution of cells in the bottle. Initial speed for the roller bottle should be 0.3-0.4 rpm. Cells will firmly attach within 12-24 hours. The speed of the roller bottle should be increased to 0.5-1 rpm after adequate inoculation. Corning's CellBind surface roller bottle is suggested to use for culture with Plus MDCK.

Microcarrier Culture

Cell cultured in low protein media are more sensitive to mechanical stress. Conditions with higher shearing force may require modifications to the culture protocol with higher density of cell inoculums. In our laboratory, a 125 ml spinner flask was inoculated at 4x10^4 cells/mg of beads with bead concentration of 5 mg/ml at recommended speed of 40-60 rpm. Total medium volume was 100 ml. We observed cells attaching on Cytodex 1 microcarriers within 10 to 30 minutes after inoculation.

BelloCell Culture

Pre-warm 450 ml culture medium at 37℃. Inoculate the bottle with about 1-1.5x10^8 cells in a 50 ml culture medium and make the total culture medium volume 500 ml. The relative volume can be adjusted such as 480 ml culture medium and 20 ml cultre medium with cells. Dispensing the cells on the top of matrix basket in order to achieve better distribution of cells on the carriers. Move the bottle on BelloStage and start Run right after dispensing the cells. Set the parameter at Up: 2.0 mm/s, Down: 2.0 mm/s, T_H: 20 s, B_H: 0 s and let run for 3~4 hours. After seeding phase, switch the parameters to Up: 1.0 mm/s, Down: 1.0 mm/s, T_H: 10 s, B_H: 10 s for cell culture. Due to the medium contains more buffers for virus production, higher CO2 concentration, e.g. 7% maybe required for the first day after inoculation if the culture medium tends to be with higher pH initially. Monitor the pH and residual glucose to decide if adjust of CO2 or medium exchange is needed. Cells will reach confluence at about 5~6 days culture.

Quality Control Testing

The performance of Plus MDCK SFM is tested for quality control using MDCK cells. pH, osmolality of the medium are measured and tested for the absence of bacterial, fungal and endotoxin contaminants.

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