HiMedia CellCulture
Enabling Breakthroughs

Product Information

BHKin1™

Serum Free BHK Medium, Animal Component Free, Chemically Defined
w/ Pluronic F-68® and Sodium bicarbonate
w/o L-Glutamine
1X Liquid Serum Free Cell Culture Medium

Product Code: SFM006L

Product description:

Serum free media are designed to grow a specific cell type or perform a specific application in absence of serum. Unlike serum supplemented media which may be utilized for a broad range of cell types and culture conditions, serum free media are highly specific.

SFM006L, serum free BHK medium is a medium designed for growth and maintenance of BHK cells. The medium is devoid of L-Glutamine.

Contents:

Code	Contents
Part A	Basal Medium, liquid
Part B	Growth Supplement

Directions:

Preparation of complete medium (SFM006L):

1. Thaw the growth supplement (Part B) overnight at 2-8°C.
2. Disinfect the external surface of bottle of Part A and Part B by spraying isopropyl alcohol before placing in a biosafety hood.
3. Transfer the entire content of one bottle of Part B to given quantity of basal medium (Part A) under aseptic conditions.
   Note: If desired, 10ml of Antibiotic-Antimycotic solution (A002) can be added to 1 litre of complete medium.
4. Add 20ml/L of 200mM L-Glutamine solution (TCL012) to the medium to obtain final concentration of 4mM L-Glutamine.
   Note: Do not mix vigorously. Doing so will cause formation of foam.
5. Store the complete medium (SFM006L) at 2-8°C until use.

Procedure for Adaptation:

BHK cells are adapted to serum free conditions by gradual weaning. Gradual weaning is a slow procedure that involves decreasing the percentage of serum in the medium, thereby gradually adapting the cells to serum free conditions.

Critical points:

- Cells used for adaptation should exhibit a healthy morphology and have more than 90% viability.
- Cells should be in the mid-logarithmic phase of growth.
- It is necessary subculture the cells at least thrice at each step, before going to the next step of adaptation.
- Subculturing should be performed when the cells are 70-80% confluent.

Gradual weaning:

Note: The procedure for gradual weaning is also applicable for adapting cells from current serum free medium to the new serum free medium.

1. Subculture the cells from serum containing medium and seed them in 75:25 ratio of serum containing medium and SFM006L with a seeding density of 0.3-0.5X10^6 cells/ml.
2. Incubate at 37°C in a humidified atmosphere with 5% CO_2. Make provision for gas exchange by loosening the caps of flasks in case of closed caps or use vented caps.
3. Subculture once the cells are 70-80% confluent.
4. Determine cell density and reseed the cells in 75:25 ratio of serum containing medium and SFM006L.
   ***Note**: It is necessary to subculture the cells at least thrice at each step of adaptation before going to the next step.*
5. Repeat steps 1 to 4 for three subcultures of each step of gradual adaptation.
   Note: Refer figure 1 for details of each adaptation step.

6. After step 3 (25:75 serum containing medium: SFM006L) of adaptation, the cells cannot be directly subcultured in 100% serum free conditions. Complete withdrawal of serum may alter cell morphology and decrease the cells viability. Hence, it is very critical to maintain them at 10:90, 5:95 and 1:99 ratios before 100% serum free conditions.
7. When the cells reach 100% serum free step of adaptation, subculture them respectively till a cell density of 1.5- 2.0X10^6 cells/ml is obtained within 4-6 days of culture. At this point, the cells are considered to be adapted to serum free conditions.

Material required but not provided:

Tissue culture grade water (TCL010)
L-Glutamine 200mM Solution (TCL012)
1N Hydrochloric acid (TCL003)
1N Sodium hydroxide (TCL002)
Trypsin – EDTA solution 1X (TCL007)
ZymeFree™ Enzyme Free Cell Dissociation Reagent (TCL028)
Trypsin Inhibitor from Soyabean 1X (TCL068)

Quality control:

Appearance
Part A: Orangish red colored clear solution
Part B: Clear colorless solution

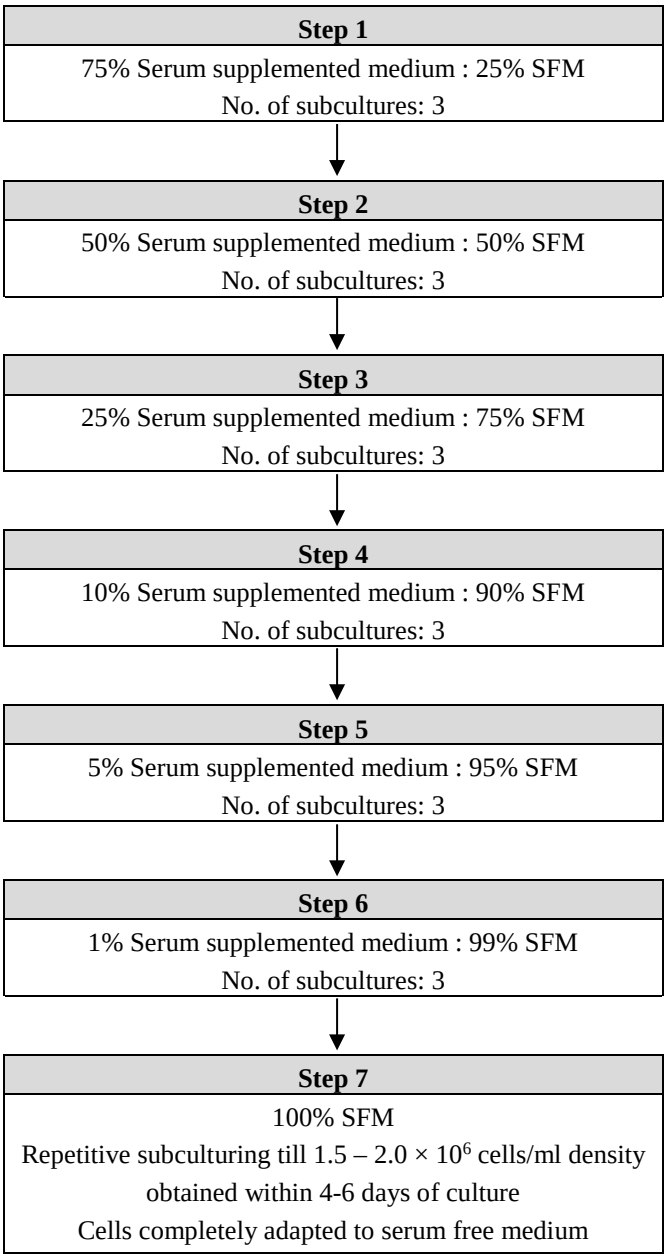
pH of Part A
7.00 - 7.60

Osmolality of Part A (mOsm/KgH_2O)
280.00- 320.00

Cultural Response
The growth promotion capacity of the medium is assessed qualitatively by analyzing the cells for the morphology and quantitatively by estimating the cell counts.

Endotoxin content
Less than 1 EU/ml

Fig 1: Gradual weaning



Storage and shelf life:

Store basal medium at 2-8ºC away from bright light.
Store serum free growth supplement at -20 ºC.
Use before expiry date given on the product label.
Shelf life of the complete medium is 8 weeks at 2-8ºC.
***Note**: Freezing of the basal medium and complete medium is not recommended. Avoid repeated freezing and thawing of the growth supplement.*

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