# CellCor<sup>™</sup> Kera CD AOF User Guide

Chemically defined & Animal origin free medium for human keratinocyte



### **Product Description**

CellCor™ Kera CD AOF is a serum-free, chemically defined culture medium for human keratinocyte growth and proliferation. It contains no animal or human extracts or lysates and is composed of synthetic or recombinant proteins. This product has been tested for bioactivity, endotoxin, mycoplasma, etc.

Product No.	Product	Volume	Storage	Shelf life
YSP019	CellCor <sup>™</sup> Kera CD AOF	500 mL	Under −20℃	12 months

### **Key Requirements**

ltem	Recommendations		
Antibiotics	Gentamicin or Penicillin streptomycin		
Detach solution	TrypLE™ Express (Gibco)		
Culture ware	Tissue Culture Flask, Plate, Cell Factory (Corning, Nunc or Falcon)		
Seeding cell counts	3,000-5,000 cells/cm <sup>2</sup>		
Media volume	T25 flask (5 mL), T75 flask (15 mL), T175 flask (30–40 mL)		
Cell harvest confluency	75-85% (Figure.1)		

※ Note: Why Use TrypLE™ Express and Not Trypsin-EDTA? CellCor™ Kera CD AOF does not contain any serum components that could stop the activity of trypsin. Therefore, we strongly recommend the use of TrypLE™ Express, which does not require serum neutralization.

# How to use CellCor™ Kera CD AOF

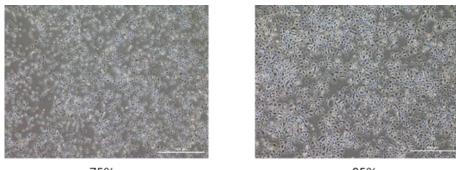
### Thawing

- Before using CellCor™ Kera CD AOF for cell culture, aliquot and warm the medium in a 37°C water bath for 30 minutes.
  ※ Use immediately after thawing. Avoid additional freeze-thaw cycles.
- 2. Add 9 mL of pre-warmed CellCor™ Kera CD AOF into a 15 mL tube.
- 3. Thaw cryovial in a 37°C water bath for 1-2 minutes.
- 4. Disinfect the surface of the cryovial with 70% ethanol and place it in the BSC.
- 5. Carefully transfer the thawed cells to the 15 mL tube containing CellCor™ Kera CD AOF.
- 6. Centrifuge the tube at 200-300 xg, RT for 3-5 minutes.
- Remove the supernatant and suspend cells with CellCor™ Kera CD AOF. Count cells and seed to flask at density <u>3,000-</u> <u>5,000 cells/cm<sup>2</sup></u>.
- 8. Incubate at  $37^{\circ}$ C in a humidified atmosphere of 5% CO<sub>2</sub>.
- 9. Proceed to sub-culture when cells reach 75-85% confluency (Figure.1)

### ■ Sub-culture

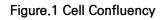
- 1. Remove the cultured medium and wash with DPBS.
- 2. Detach cells using TrypLE™ Express and recover to CellCor™ Kera CD AOF.
- \* Use a microscope to confirm that the cells have completely detached from the surface of the culture vessel.
- 3. After centrifugation at 200–300xg, RT, 3–5 minutes, the cell pellet is resuspended in CellCor™ Kera CD AOF to count cells.

4. Seed the cells at <u>3,000-5,000 cells/cm<sup>2</sup></u> and grow to confluence <u>75-85%</u> (Figure.1) in a 37°C, 5% humidified CO<sub>2</sub> incubator.









## FAQ

### 1. Why do Adaptation step is required?

Adaptation may be required to minimize the effects of previously used establishment or culture media and to ensure stable cell attachment and culture under serum-free chemically defined medium conditions (optional).

#### Adaptation (optional)

- 1. After 24 hours of thawing or sub-culturing the cells with the existing medium, remove half of the culture medium and fill half with CellCor<sup>™</sup> Kera CD AOF.
- 2. When cell confluency reaches 75-85% (about 3-4 days), sub-culture using only CellCor™ Kera CD AOF.

#### 2. Is there a recommend flask or coating reagent?

No coating is required when using standard tissue culture flasks (Corning, Nunc, Falcon products).

#### 3. Dose the cell detachment solution has to TrypLE Express?

Serum-free media does not contain serum components, which can damage cells when using products with high trypsin activity. We recommend the use of TrypLE™ Express, which minimizes cell damage.

#### 4. <u>Are there any precautions when using CellCor™ Kera CD AOF after thawing?</u>

Repeated freezing-thawing cycle is not recommended, and if used as aliquot, it can be used for up to 4 weeks at 4°C within the expiry date.