

# Product Instruction

## PK15 Cell Serum-free Medium

### Product Type: Proli-S001

#### Product Description

Proli-S001 is a serum-free medium developed by Shanghai BioEngine Sci-Tech Co., Ltd., which is designed based on the growth and metabolism characteristics of PK15 cells. This medium is suitable for high-density suspension culture of PK15 and high-efficiency production of porcine circovirus (PCV).

#### Product Formula

The intellectual property rights of PK15 cell serum-free medium formula are owned by Shanghai BioEngine Sci-Tech Co., Ltd.

#### Product Ingredient

The medium contains carbohydrates, amino acids, vitamins, metal ions and other nutritional components.

This product does not contain components of animal origin, genetically modified plant origin or raw material with mad cow virus origin.

#### Product Preservation

- Store in a dark environment at 2-8°C.
- It is recommended to use within two weeks after opening.

#### General culture recommendations

##### Subculture

➤ The recommended seeding density is  $0.8-1.2 \times 10^6$  cells/ml for suspension culture. Subculture cells every 48 hours.

➤ Incubate the cells at 37°C in a suitable incubator with 5% CO<sub>2</sub> in air atmosphere. Use shake flasks with vent cap. And the recommended shaker speed is 110-130 rpm.

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### **Cryopreservation**

Prepare the desired quantity of cells, harvesting in mid-log phase of growth with good condition. The recommended cryopreservation density is  $2.5-3.5 \times 10^7$  cells/ml/vial. And the cryopreservation medium is prepared as 93% (v/v) fresh medium supplemented with 7% (v/v) DMSO. Centrifuge cells at  $190 \times g$  for 5 min and discard the supernatant. Resuspend pellets with cryopreservation medium and dispense aliquots of this suspension into cryovials with 1 ml/vial. Achieve cryopreservation in an automated controlled rate freezing container and keep it in  $-80^\circ\text{C}$  freezer overnight. Transfer frozen cells to liquid nitrogen for long-term storage.

### **Recovery**

Thaw the vial by gentle agitation in a  $37^\circ\text{C}$  water bath. This process should be rapid (approximately 2 minutes). Remove the vial from the water bath as soon as the contents are thawed. All the operations from this point on should be carried out under strict aseptic conditions. Transfer the vial contents to a centrifuge tube with 10 ml of growth medium and centrifuge cells at  $190 \times g$  for 5 min to wash away DMSO. Discard the supernatant and resuspend the pellets with 20-30 ml of growth medium into a 125 ml shake flask. The recommended seeding density is  $0.8-1.2 \times 10^6$  cells/ml.