

# Product Instruction

## Proli-S001S PK15 Serum-free Medium

### Product Type: Proli-S001S

#### Product Description

PK15 serum-free medium is a serum-free, non animal-derived base medium with clear chemical composition, which is independently developed, researched and produced by Shanghai BioEngine Sci-Tech Co., Ltd. This medium is suitable for high-density culture and efficient proliferation of PK15 cells, and supports the efficient expansion of circovirus.

#### Product Formula

The intellectual property rights of Proli-S001S PK15 serum-free medium formula are owned by Shanghai BioEngine Sci-Tech Co., Ltd. For additional information, please contact our technical support department.

#### 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.

This product is a completely serum-free, without the addition of serum or plasma.

#### Product Preservation

- Store in a dark environment at 2-8°C.
- This product is vulnerable to water damage. Please use immediately after opening. If it needs to be stored, please sealed by heat sealing and sealing clips, avoiding damp and being ineffective.
- Do not recommend to use, when the product is beyond expiration date.

#### Instruction for the preparation of PK15 serum-free medium

Do the preparation of PK15 medium as per the one Table 1 shows

Component	Concentration
Dry powder of Proli-S001S	27.15 g/L
Sodium bicarbonate	2.00 g/L

**Table 1 Formula table of PK15 medium**

(1) Weigh 100% water of the final medium preparation volume into the medium preparation container. When preparing, ultrapure water or water for injection and above standard water should be used, and the water temperature should be controlled at 28-32°C.

(2) Turn on the mixing system of the medium preparation container, stir thoroughly, and avoid the generation of air bubbles during stirring.

(3) Accurately weigh 27.15 g/L of dry powder of Proli-S001S, and add them into the preparation container near the liquid surface or use special equipment such as homogenizer, and stir thoroughly for 20-30 min.

(4) Slowly add, dropwise, 5 mol/L sodium hydroxide solution to the solution prepared in step (4), and adjust its pH value to 6.1-6.6, and stir thoroughly for 10-20 min. The recommended addition amount of sodium hydroxide is 0.25 g/L.

(5) Accurately weigh 2.00 g/L sodium bicarbonate powder, and add them into the preparation container near the liquid surface or use special equipment such as homogenizer, and stir thoroughly for 10-20 min.

(6) Use 1mol/L hydrochloric acid solution to adjust the pH value of the medium to 7.0-7.4 (If necessary).

(7) Use a pulse pump or compressed air (3-15 psi) to sterile filter the Proli-S001S medium solution through a sterile filter membrane with 0.22 μm pore size.

(8) The prepared medium liquid should be stored in a dark environment at 2-8 °C , and the expiration date is one month.

(9) The reference parameters of product

Indicator	Reference Standard
Product initial pH value	4.50-4.90
Osmolality	300-350 mOsm/Kg
Product turbidity	<4.00NTU

**Notes:**

(1) The above units of "g/L" are volume concentration (solute weight/solution volume).

(2) The above preparation parameters (such as stirring time, etc.) are for the reference of small-scale preparation in research and development. When in production, please set appropriate preparation parameters according to the stirring capacity of the preparation vessel.

(3) The product belongs to carbon dioxide buffer system. The product final pH value may rise when vigorous stirring or long-time stirring, which is a normal phenomenon and does not affect the use of the product.

## Medium Usage

### Passaging cells

- PK15 cells that have been suspended in other serum-free medium can be directly centrifuged and replaced with Proli-S001S serum-free medium.
- For adherent cultured cells, it is recommended to habituate the adherent cells according to the content in the "Cell Habituation" section of this instruction, and then use Proli-S001S serum-free medium for suspension subculture.

Recommendation: The seeding density should be controlled at  $0.8-1.2 \times 10^6$  cells/ml during serum-free suspension subculture, and subculture should be carried out every 48 hours.

### Cryopreservation

Select the cells that are in good condition in the logarithmic growth phase for cryopreservation. The cryopreservation density is  $2.5-3.5 \times 10^7$  cells/ml/tube. The ratio of cryopreservation solution is 45% culture supernatant + 45% fresh medium + 10% DMSO. Centrifuge 175 g cells for 5 min, discard the supernatant, resuspend the mixed freezing solution, and dispense the cells into freezing tube, 1 ml/vial. Then, place them in a programmed cooling box at  $-80^{\circ}\text{C}$  overnight, and transfer to liquid nitrogen save

### Recovery

Rotate the freezing tubes in the same direction in a  $37^{\circ}\text{C}$  water bath to quickly melt the cryopreservation solution, and take them out to a clean bench when only small ice crystals remain. Add 10 ml of culture medium and 175 g cell to the centrifuge tube, centrifuge for 5 min, and wash away the DMSO. Use 20-30 ml of medium to resuspend the cells into a 125 ml shake flask with a vented cap, and control the seeding density at  $0.8-1.2 \times 10^6$  cells/ml.