

# **Prol-S001S PK15 Serum-free Medium**

**Product Name: Prol-S001S**

## **User Manual**

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## Description

Prol-S001S is a serum-free culture medium developed by Shanghai Biotechnology Co., Ltd. targeting the growth and metabolism characteristics of PK15 cells. It is protein free and animal component free, suitable for high-density suspension culture of PK15 cells, and supports efficient production of porcine circoviruses.

## Application

This product is intended for research or further manufacturing in the bio-manufacturing industry, but not for human or therapeutic use.

## Composition

The IP rights of Prol-S001S medium formulation are owned by Shanghai BioEngine Sci-Tech Co., Ltd.

This medium contains:

- Carbohydrates, amino acids, vitamins, bulk salts, and trace elements.
- 8.3 g/L D-glucose, 2 g/L P188, 8 mM glutamine.

Not contain:

- Cytokines, antibiotics, HEPES and phenol red.
- Raw materials from animal sources.

## Storage

- Store medium at 2-8°C, away from light.
- Once opened, the powder medium should be stored protected from moisture in a tightly sealed container.
- Do not use it after the expiration date or being damped.

## Reconstitution of Powder Medium

Table 1 shows the preparation of Prol-S001S medium [1].

Ingredients	Concentration
Prol-S001S medium powder	27.15 g/L [2]
Sodium bicarbonate	2.00 g/L

Table 1. Preparation of Prol-S001S medium

- 1) Weigh 100% water of the final volume into the preparation container using pure water, ultrapure water, or water for injection at 28-32°C. Mix thoroughly without creating air bubbles.
- 2) Accurately weigh the corresponding mass of Prol-S001S medium at a concentration of 27.15 g/L and add it into the preparation container of 1) step. Stir well for 20-30 minutes.
- 3) Slowly adjust to pH 6.0-6.5 with 5-10 mol/L sodium hydroxide solution. The recommended amount of sodium hydroxide is 0.25 g/L. Stir for 10-20 minutes. At this point, the solution should be clear.
- 4) Weigh 2.00 g/L of sodium bicarbonate powder, add it slowly near the liquid level in the container, and stir for 10-20 minutes.

- 5) Adjust to pH 7.0-7.4 with sodium hydroxide or hydrochloric acid solution if the pH is beyond this range.
- 6) Pass the medium solution through a pore size of 0.22 or 0.2 µm sterile filter membrane, such as PES, using a pulse pump or compressed air (3-15 psi).
- 7) Use the prepared medium liquid immediately or store it in glass bottles, PET storage bottles, or disposable storage bags with an oxygen barrier membrane in a dark environment of 2-8°C. It's recommended for use within one month.

**Note:**

<sup>[1]</sup> The above parameters (such as stirring time) are set for small-scale liquid preparation. Adjust these parameters for large-scale preparation based on container capacity to ensure full dissolution of dry powder.

<sup>[2]</sup> The “g/L” unit denotes volumetric concentration (solute mass/ water volume).

Specifications of final liquid medium

Test	Unit	Specification
pH		7.0 – 7.4 <sup>[3]</sup>
Osmolality	mOsm/kg	300 – 350
Turbidity	NTU	< 4.00

Table 2. Specifications of final liquid medium

**Note:**

<sup>[3]</sup> The pH buffer system of the product is carbon dioxide-sodium bicarbonate. The final pH value should be strictly controlled within the specific range outlined in Table 2. The following operations, such as prolonged reconstitution time or aeration in the bioreactor without pH control, can result in a gradual pH increase. There is a risk of metal ion precipitation when the pH value exceeds the upper limit.

**Cryopreservation**

- 1) Harvest cells in the mid-log phase of growth with >90% viability by centrifugation at 190×g for 5 minutes.
- 2) Prepare cryopreservation medium with 93% Proli-S001S medium and 7% DMSO on the day of use.
- 3) Resuspend cells in cryopreservation medium to a final viable cell density of 2.5-3.5×10<sup>7</sup> cells/mL or as required.
- 4) Dispense aliquots of the cell suspension into cryovials.
- 5) Achieve cryopreservation in an automated or manual controlled rate freezing apparatus (0.5-1°C decrease per minute is suggested).
- 6) Transfer frozen cells to liquid nitrogen storage.

**Cell Recovery**

- 1) Rapidly thaw frozen cells in a 37°C water bath. Transfer to a super clean bench as soon as melted or with small ice crystals.
- 2) Transfer the vial content into a centrifuge tube containing 10 mL of prewarmed Proli-S001S medium. Harvest the cells by centrifugation at 190×g for 5 minutes and discard the supernatant.
- 3) Resuspend cells by prewarmed Proli-S001S medium to a viable cell density of 0.8-1.2×10<sup>6</sup> cells/mL in a 125mL shake flask.
- 4) Incubate the shake flask at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in air on an orbital shaker platform rotating at 110-130 rpm (110 rpm for 50 mm amplitude; 130 rpm for 10 mm amplitude).
- 5) Cells should be sub cultured and adapted at least two passages. After the cell specific growth rate (or doubling time) reaches stability, subsequent operations can be carried out.

## Subculture Cells

- 1) Ensure that the cell viability is >90%, and the growth rate is in mid-logarithmic phase prior to subculturing.
- 2) Calculate the volume of cell culture and prewarmed medium necessary to seed at  $0.8-1.2 \times 10^6$  viable cells/mL in a shake flask.
- 3) Incubate at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in air on an orbital shaker platform rotating at 110-130 rpm (110 rpm for 50 mm amplitude; 130 rpm for 10 mm amplitude).
- 4) Subculture cells every two days according to the above steps.

## Related Product

Product	Cat. No.	Form	Size	Packaging	Notes
Prol-S001S Serum-free Medium	PK15	EXP0111101	Powder	200 L Bag	● SF, PF, ADCF
		EXP0111102	Powder	100 L Bag	● Supports efficient production of the porcine circovirus
		EXP0111103	Powder	10 L Bag	



Scan the QR code for more product information.

Stay tuned for more updates.

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