

Document: ProdSpec PP01-03
Created on: 24 JUN 2025
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Last approved by: Julian Arjuna Bisten

Product Specification Sheet

Proliferum[®] P Panel

Serum replacements for cell culture media address the scalability, performance, sustainability and ethical challenges of foetal bovine serum (FBS). However, developing effective alternatives has traditionally been costly and time-consuming. This delays new applications of cell culturing in getting from lab to market.

At Multus, we make development of animal component-free (**ACF**) media cheaper and faster, delivering affordable, high-performance media for a wider variety of cells. Our products are designed to help establish scalable processes for animal cell culturing.

Our **panel of 3 formulations** is designed to support the growth of porcine adipose derived stem cells.

Functional Profile

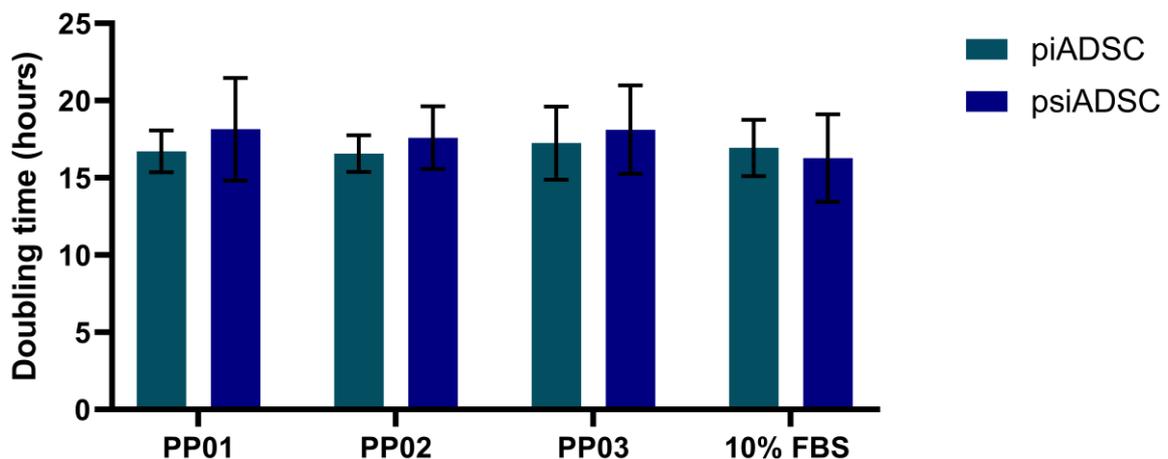


Figure 1: Average doubling times from 5 passages between 10% FBS and 10% (1X) Proliferum[®] P in DMEM/F12. The cell models tested are piADSCs (porcine immortalised adipocyte derived stem cells-genetically engineered) and psiADSCs (porcine spontaneously immortalised adipocyte derived stem cells).

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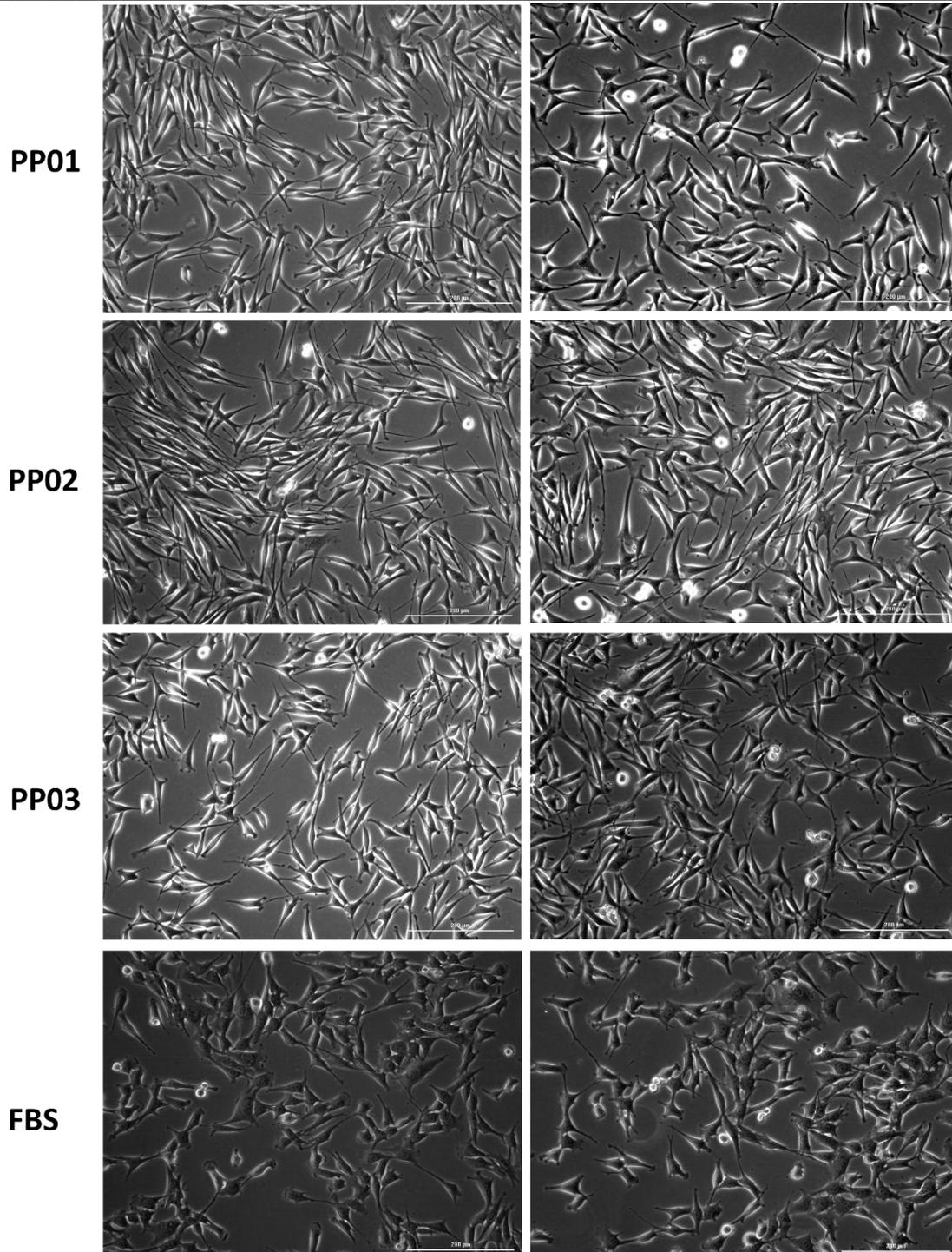


Figure 2: Morphology of piADSCs (left column) and psiADSCs (right column) porcine cells after 5 passages in media supplemented with PP01, PP02, PP03, or 10% FBS.

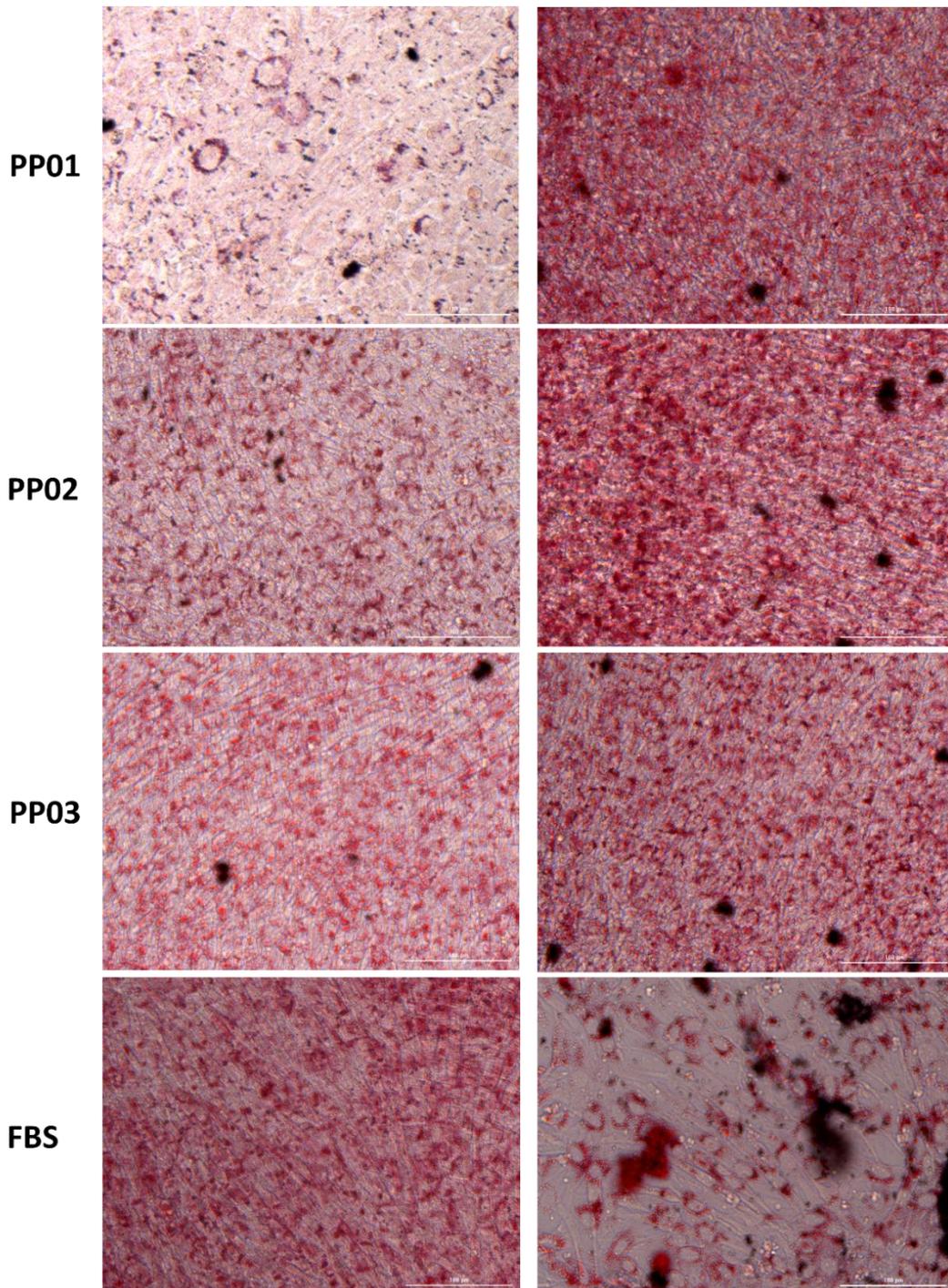
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Figure 3: Adipose differentiation test on piADSCs (left column) and psiADSCs (right column) porcine cells after 3 passages in media supplemented with PP01, PP02, PP03, or 10% FBS.

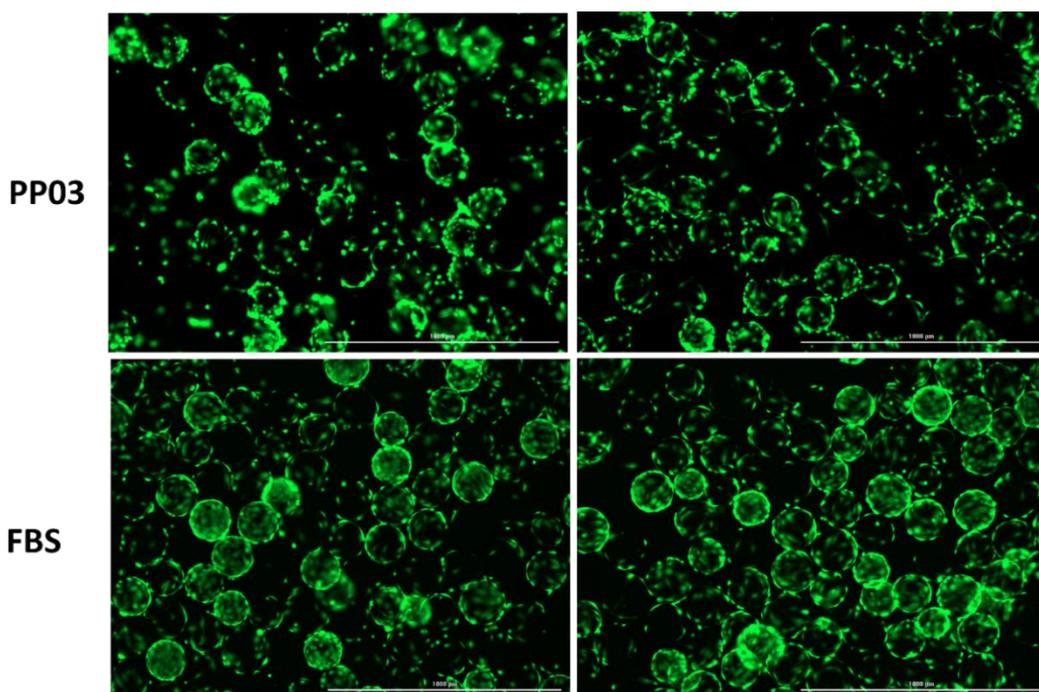


Figure 4: Live (green) / dead (red) cell staining on piADSCs (left) and psiADSCs (right) porcine cells after culture in microcarriers on Cytodex III media supplemented with PP03 or 10% FBS.

Quality Control

| Test | Specification |
|-------------------------|---------------|
| pH | 6.5 - 8.5 |
| Osmolarity (mOsm/kg) | 290 - 320 |
| Bacterial Testing | Negative |
| Fungal Testing | Negative |
| Mycoplasma Testing | Negative |
| Particulate Examination | Negative |
| Cell Growth | Pass |

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Table 1: *Quality Control (QC) tests and their specifications.*

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Usage Instructions

- **Cell Culture Surface Coating:** We recommend using a cell type suitable coating (**Gelatin, Vitronectin, Laminin, etc.**) for optimal results. Coating is particularly important when switching from serum-containing to serum-free media.
- **Storage:** Upon arrival, store at **-20°C** and minimise light exposure.
- **Thawing:** Thaw at room temperature (**25°C**). Thawing at 37°C may impact shelf life.
- **Filtering:** The product is manufactured under **sterile** conditions. Further filtering (0.2 or 0.4 micron) may impact product performance.
- **Aliquoting and Shelf Life:** Thaw only the amount of serum needed for 1-2 weeks. Make the complete media immediately upon thawing. Store it at **2-8°C** and use it within **1-2 weeks**. Refreezing serum or complete media at -20°C may impact shelf life.
- **Complete Media Preparation:** For cell culturing dilute in basal media (DMEM/F12 recommended) to your desired concentration. We recommend 1-2X but suggest titration experiments to determine the optimal concentration for your cells. Ensure the complete media has reached 25°C prior to cell culturing and minimise light exposure.

| Proliferum® P Dilution Chart | | |
|------------------------------|------------------------|------------------------|
| | 1L Proliferum® P (1X) | 1L Proliferum® P (2X) |
| Usage | Replaces 1L of 10% FBS | Replaces 1L of 20% FBS |
| Proliferum® P (10X) | 100mL | 200mL |
| DMEM/F12 | 900mL | 800mL |

Table 2: Dilution chart to make up 1L of 1X or 2X complete medium to substitute 10% or 20% FBS.

- **pH and Osmolarity:** The pH and osmolarity may change when combined with basal media. If you are working with cell lines that are sensitive to osmotic shock, adjust the complete media with 5M NaCl or other salt solutions.
- **Media Exchange:** We recommend exchanging every **48h**.

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- **Sequential Media Adaptation:** Switching directly from FBS to Proliferum[®] P is recommended (with an appropriate tissue culture coating). If cells exhibit poor viability and/or growth, we recommend the following adaptation protocol:

| Proliferum[®] P Sequential Adaptation Plan | |
|--|---|
| Passage 1 | 50% FBS medium : 50% Proliferum [®] P medium |
| Passage 2 | 25% FBS medium : 75% Proliferum [®] P medium |
| Passage 3 | 0% FBS medium : 100% Proliferum [®] P medium |

Table 3: Passage-by-passage sequential adaptation plan.

- **Cell Passaging:** When passaging cells for serum-free culture, we recommend the use of a **Defined Trypsin Inhibitor** (DTI; Fisher Scientific #10703864) to inactivate the trypsin.
- **Regulatory Information:** It is an animal component free (**ACF**) media. We are working on replacing the 2 proteins that currently still contain **human sequences**.