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Created on: 11 NOV 2024
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Created by: Julian Arjuna Bisten
Last approved by: Julian Arjuna Bisten

Product Specification Sheet
Proliferum® B 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 4 formulations** is designed to support the growth of various bovine and murine cell types, performing best on fibroblasts.

Functional Profile

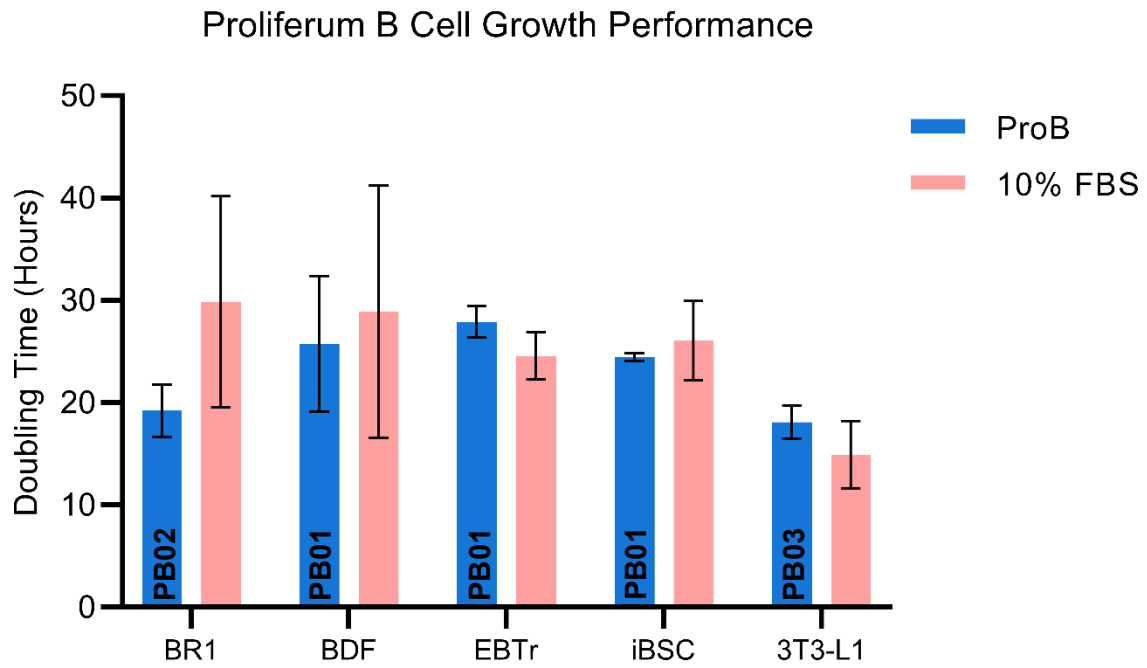

Figure 1: *Average doubling times from ≥ 3 passages (n=2) between 10% FBS and 10% (1X) Proliferum® B in DMEM/F12. For each cell model, the best performing formulation from the panel is presented.*

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Table 1: *Table of cell models indicating cell line description and best performing panel formulation.*

Cell Model	Description	Best Performing Proliferum® B
BR1	Primary **bovine** fibroblast isolated in-house from muscle tissue	**PB02**
BDF (SC-B2300)	Primary **bovine** fibroblast isolated from foetal skin	**PB01**
EBTr (BNL-4) (ATCC CCL-44)	Immortalised **bovine** fibroblasts derived from the trachea of a male embryo	**PB01**
iBSC (ETU009)	Immortalised **bovine** satellites from Tufts University	**PB01**
3T3-L1 (CL-173)	Immortalised **murine** fibroblast/pre-adipocyte	**PB03**

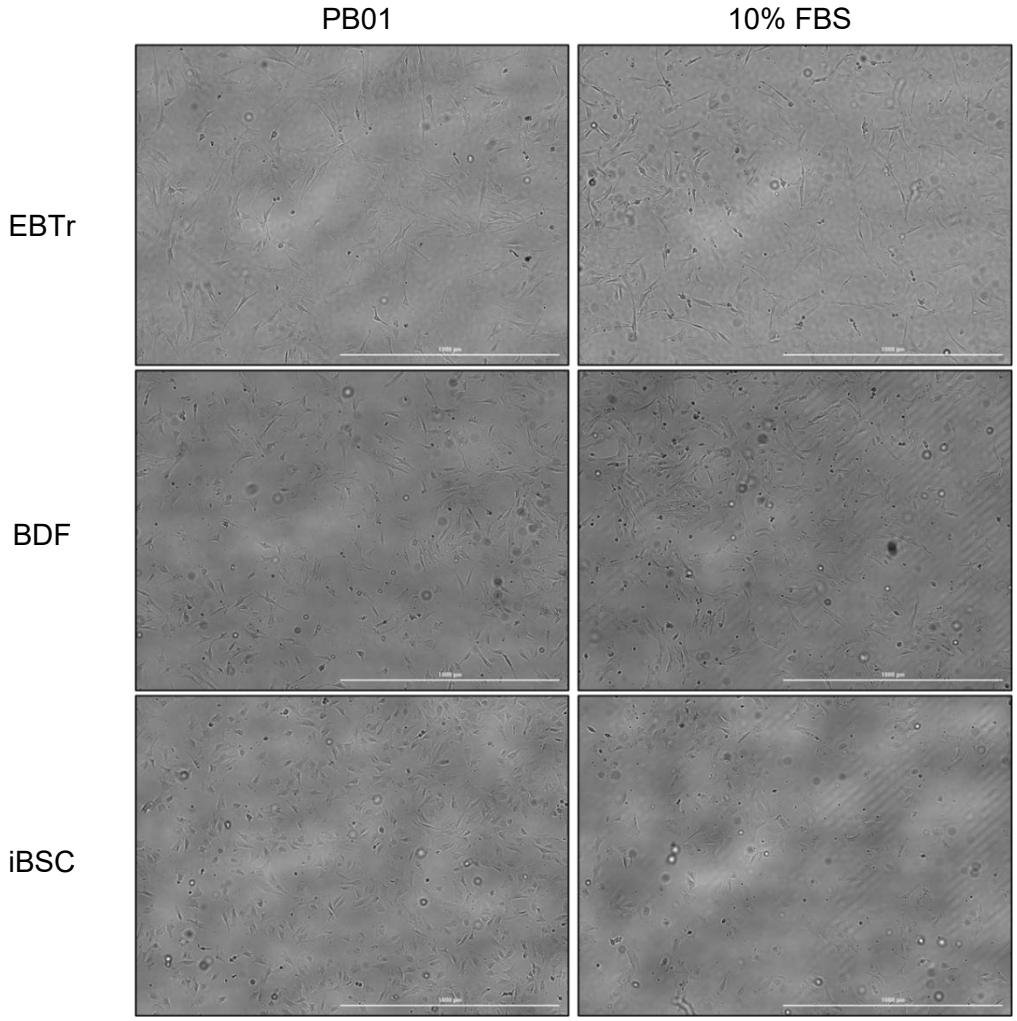

Figure 2: *Morphology of EBTr, BDF and iBSC bovine cells after two passages in media supplemented with PB01 (left column) and 10% FBS (right column).*

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Quality Control

Table 2: *Quality Control (QC) tests and their specifications.*

Test	Specification
pH	7.0 - 9.5
Osmolarity (mOsm/kg)	280 - 320
Bacterial Testing	Negative
Fungal Testing	Negative
Mycoplasma Testing	Negative
Particulate Examination	Negative
Cell Growth	Pass

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**) in the dark. 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.

Table 3: *Dilution chart to make up 1L of 1X or 2X complete medium to substitute 10% or 20% FBS.*

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

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

Table 4: *Passage-by-passage sequential adaptation plan.*

Proliferum® B Sequential Adaptation Plan	
Passage 1	50% FBS medium : 50% Proliferum® B medium
Passage 2	25% FBS medium : 75% Proliferum® B medium
Passage 3	0% FBS medium : 100% Proliferum® B medium

- **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 4 proteins that currently still contain **human sequences**.