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**Created by:** Julian Arjuna Bisten

**Last approved by:** Julian Arjuna Bisten

**Department:** Production

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## Product Specification Sheet

### Vitronectin

A critical challenge in the cell culture industry is the cost of growth media. Current solutions are not designed for the food industry. FBS is often used in cell culture but is unethically sourced and has large price and performance fluctuations. Serum-free formulations are typically expensive, do not perform well across the range of cell lines used in cultivated meat, and are not designed for scale.

Multus creates the key ingredients for companies to accelerate R&D and scale production to bring cultivated meat to market affordably and profitably.

One of the key ingredients commonly used in both the biopharmaceutical and cellular agriculture industries is Vitronectin. Multus produces recombinant human Vitronectin which can be used as an extracellular matrix (ECM) to promote cell proliferation and supports normal colony morphology for different cell types.

#### Storage and Handling

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Upon arrival, store the product below  $-70^{\circ}\text{C}$ . Upon first thawing, aliquot it to avoid damage via repeated freeze-thaw cycle. Before use, thaw Vitronectin overnight at  $4^{\circ}\text{C}$ , then freeze it immediately after use.



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## Quality Controls

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Concentration: 150-250 µg/mL – for exact concentration see the side of the bottle.

Test	Specification
Bacteria Testing	Negative
Mycoplasma Testing	Negative
Fungal Testing	Negative
Particulate Examination	Negative
Endotoxin	< 2 EU/µg
Filtered	0.2 µm
Cell growth	Pass

## Identification

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The Vitronectin produced by Multus is a truncated version of the wild-type human Vitronectin (VTN-N) fused with a 6-histidine tag with amino acid sequence of:

MDQESCKGRCTEGFNVDKCCQCDLCSYYQSCCTDYTAECKPQVTRGDVFTMPEDYTVYDDGEEKNNATVHEQVGGPSLTS  
DLQAQSKGNPEQTPVLKPEEEAPAPEVGASKPEGIDSRPETLHPGRPQPPAEELCSGKPFDAFTDLKNGSLFAFRGQYCYELD  
EKAVRPGYPKLIRDVWGIEGPIDAAFTRINCQKTYLFKGSQYWRFDGVLDPDYPRNISDGFDPDNVDAALALPAHSYSGR  
ERVYFFKGKQYWEYQFQHQPSEQEEGSSSAVFEHFAMMQRDSWEDIFELLFWGRTSAGTRQPQFISRDWHGVPGQVDAA  
MAGRIYISGMAPRPSLAKKQFRHRNRKGYRSQRGHSRGRNQNSRRPSRHHHHHH

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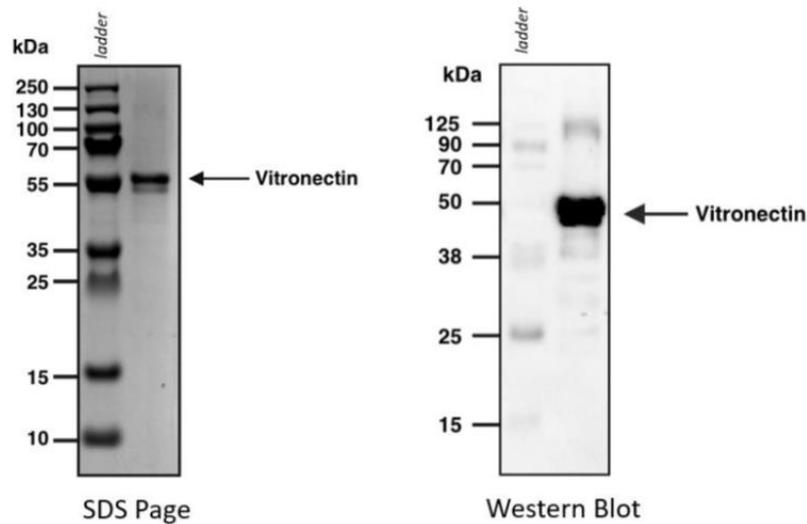


Figure 1: SDS Page and Western blot showing the purified vitronectin.

### Functional Profile - using it for coating

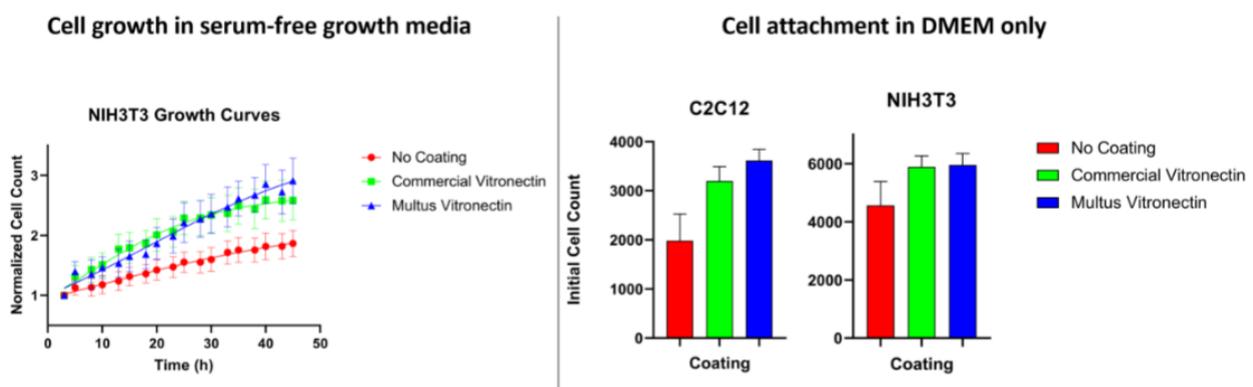


Figure 2: Performance comparison between commercial, Multus vitronectin and no vitronectin in DMEM/F12.



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## Recommendation for use

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Adapt cells to the growth media of choice for at least 48 hours before starting the experiment. Thaw Vitronectin overnight at 4°C.

For coating, dilute Vitronectin into PBS1X at a final concentration of 10 µg/ml. To coat the surface, transfer 156.25 µl/cm<sup>2</sup> of the Vitronectin solution to the centre of area (e.g., for a 96 well plate, 0.32 cm<sup>2</sup>, add 50 µl) and rock the plate/dish gently horizontally, side to side and forward-backward to spread the coating solution across the entire well surface. Incubate the plates for 1 hour at 37°C in a humidified CO<sub>2</sub> incubator. Remove Vitronectin solution from the wells and quickly rinse once with 100 µl/well of PBS1X.

After coating, add 255 µl/well of serum free medium without allowing the wells to dehydrate. Seed cells at a density of 5,000 cells/well in serum free media and grow cells at 37°C in a humidified CO<sub>2</sub> incubator.

For serum-free growth media, we recommend 10% Proliferum M in DMEM/F12.