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Certificate of Analysis

Caf1-Vitronectin

Product number: MB_002
Batch number: 15
Date of manufacture: Feb 2024
Approved by: Helen Waller
Department: Production

Caf1 is a bacterial protein that associates into long [1], flexible and bioinert [2] polymers. Bioactivity can be imparted by genetically encoding bioactive motifs into the protein's sequence [2–6]. Caf1 polymers are highly stable [7], modular and flexible.

Caf1-Vitronectin mimics the action of the extracellular matrix protein vitronectin and is adhesive for a range of cell types such as human mesenchymal stromal cells (MSCs) and induced pluripotent stem cells (iPSCs).

Cells grown on Caf1-Vitronectin display adherence and morphologies similar to those seen on recombinant vitronectin and GelTrex™.

Storage and Handling

The stock solution concentration is 1 mg/mL in water. For long term storage use -15 to -25 °C. For short term can be stored at 4 °C for up to 2 weeks. Freeze/thaw – tested up to 10 times with no effect on performance. Prepare working solutions fresh. It is **strongly not recommended** to store diluted stocks.

Quality Testing

Test	Result
Bacterial growth	Negative
Fungal growth	Negative
Particulate examination	Negative
Endotoxin level	< 40 EU/µg

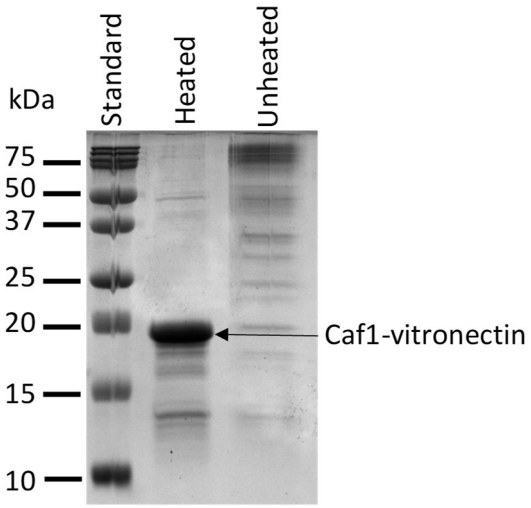

Figure 1: SDS-PAGE showing purified Caf1-Vitronectin, heated (monomer) and unheated (polymer).

Functional Assays

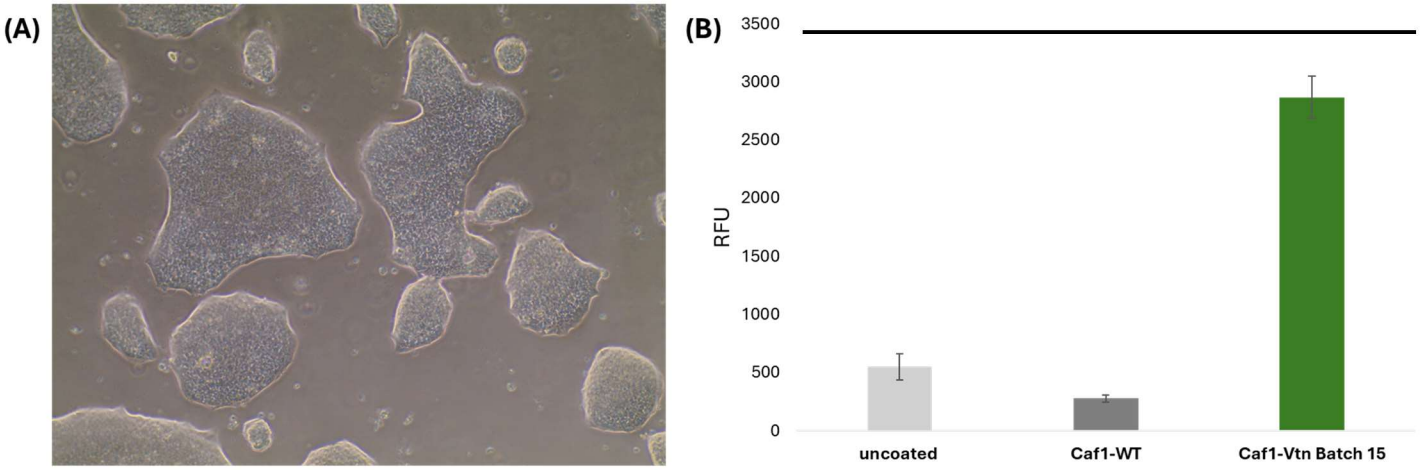

Figure 2: (A) iPSCs derived from human dermal fibroblasts were cultured on non-TC plastic culture dishes coated with 2µg/cm^2 Caf1-Vtn. Images were taken at 10X magnification, 48 hours after seeding, by bright field microscopy. **(B)** Non-TC plastic was either left uncoated, coated with Caf1-WT or Caf1-Vtn batch 8 prior to seeding hDfa iPSCs in E8 Flex media supplemented with 10 µM ROCK inhibitor. After 24 hours media was exchanged to remove the inhibitor. A further 24 hours later, cell viability was measured using the Presto Blue cell viability assay, as per manufacturer's specifications.

Protocol

Coating protocol:

1. Defrost Caf1-Vitronectin on the bench at room temperature or by placing the vial in a water bath at 37 °C. The Caf1 solution can be used at room temperature.
2. Work inside a sterile cell culture hood using sterile equipment and solutions.
3. Prepare working solution - Dilute Caf1-Vitronectin in sterile 1XPBS to a final concentration of 2-3 µg/cm^2 depending on the size of the well/dish.
4. Transfer the solution to the centre of the well. For 96 well plate use 50 µl, 48 well plate use 150 µl, 24 well plate use 300 µl, 12 well plate use 600 µl, 6 well plate use 1000 µl, for 35 mm dishes use 1000 µl, and for 60 mm dishes and T25 flasks use 2 ml to ensure full coverage of the surface.
5. Rock the plate side to side and front to back to ensure the entire well surface is coated.
6. Incubate the plate for ≥1 hour at room temperature inside the hood with the lid on.
7. Prepare cells in cell culture medium to the required cell density.
8. Aspirate the remaining Caf1-Vitronectin solution from the wells.
9. Seed cells directly onto the coated surface at the required density and grow at 37 °C in a CO_2 cell culture incubator.

References

1. Soliakov A, Harris JR, Watkinson A, Lakey JH. The structure of Yersinia pestis Caf1 polymer in free and adjuvant bound states. Vaccine. 2010 Aug 9;28(35):5746–54.
2. Roque AI, Soliakov A, Birch MA, Philips SR, Shah DSH, Lakey JH. Reversible non-stick behaviour of a bacterial protein polymer provides a tuneable molecular mimic for cell and tissue engineering. Adv Mater Deerfield Beach Fla. 2014 May;26(17):2704–9, 2616.
3. Peters DT. Engineered mosaic protein polymers; a simple route to multifunctional biomaterials. J Biol Eng [Internet]. 2019; Available from: http://doi.org/10.1186/s13036-019-0183-2
4. Dura G, Peters DT, Waller H, Yemm AI, Perkins ND, Ferreira AM, et al. A Thermally Reformable Protein Polymer. Chem. 2020 Nov;6(11):3132–51.
5. Dura G, Crespo-Cuadrado M, Waller H, Peters DT, Ferreira AM, Lakey JH, et al. Hydrogels of engineered bacterial fimbriae can finely tune 2D human cell culture. Biomater Sci [Internet]. 2021; Available from: https://doi.org/10.1039%2Fd0bm01966f
6. Le Bao C, Waller H, Dellaquila A, Peters D, Lakey J, Chaubet F, et al. Spatial-Controlled Coating of Pro-Angiogenic Proteins on 3D Porous Hydrogels Guides Endothelial Cell Behavior. Int J Mol Sci. 2022;23(23).
7. Ulusu Y, Dura G, Waller H, Benning MJ, Fulton DA, Lakey JH, et al. Thermal stability and rheological properties of the 'non-stick'Caf1 biomaterial. Biomed Mater. 2017;12(5):051001.