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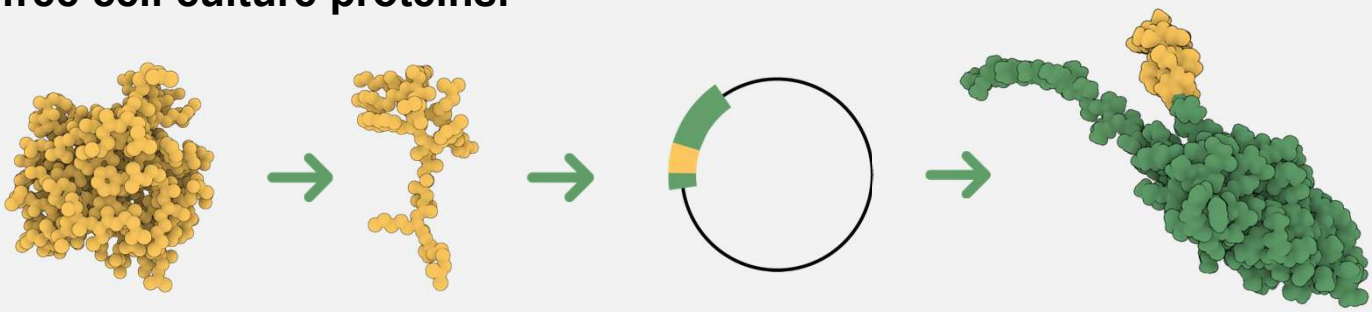
Building Better Biology

LaminB4-Cytocaf

Information Document

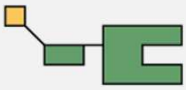
Cytocaf

Ultra stable, animal component free cell culture proteins.



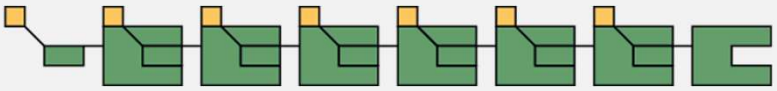
Cytocaf proteins are **engineered bacterial proteins** that are able to mimic traditional cell culture proteins through the inclusion of small, genetically encoded bioactive peptides.

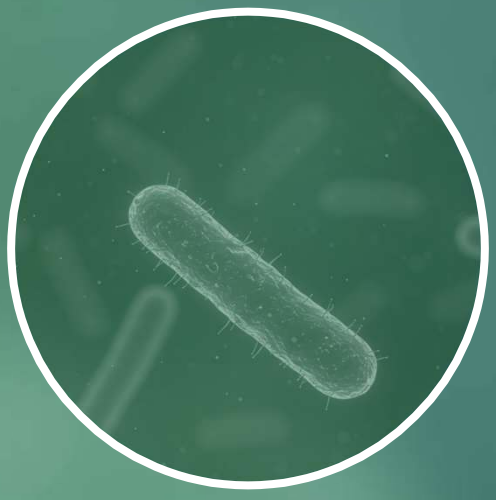
As recombinant proteins, Cytocaf proteins can be precisely defined and characterised.



The Cytocaf proteins are small, modular subunits that form long (MDa, µm) flexible polymers.

The polymers are non-covalently linked, but very highly stable, with melting temperatures >80°C making them easy to ship, store and handle at both room temperature and 37°C. There are no complex drying protocols and no need to remove lids to dry plates, reducing contamination risk.





Cytocaf proteins are expressed in **standard *E.coli* strains** in high yield, and purified by a scalable process, that uses the large size of the polymers to efficiently separate them from host cell proteins and other impurities.

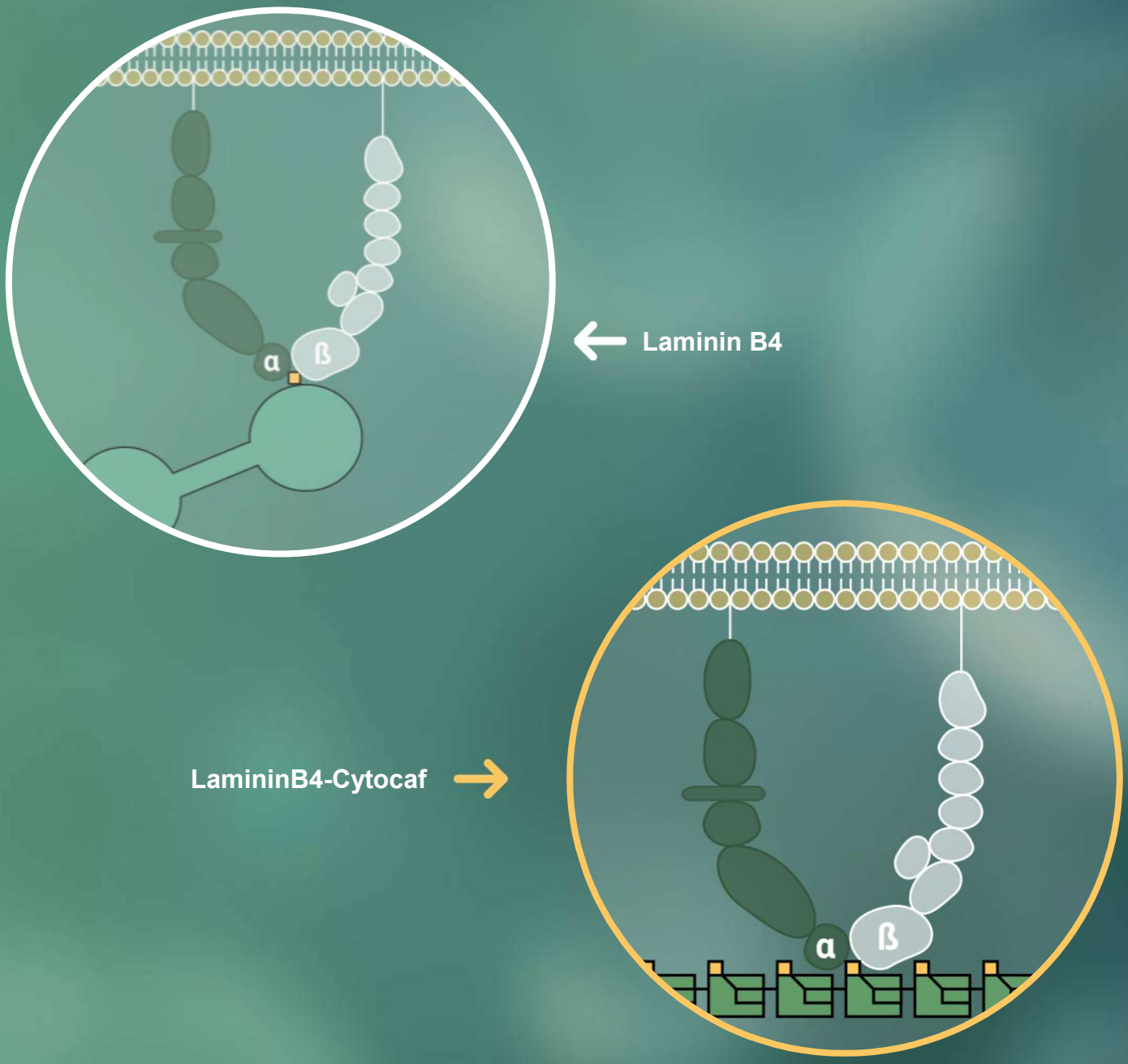
This means Cytocaf can be deployed for any cell culture process, regardless of scale.

LamininB4-Cytocaf

An ideal growth substrate for adherent cells.

LamininB4-Cytocaf is a Caf1 based protein polymer that contains a small bioactive peptide fragment from the extracellular matrix protein Laminin – specifically from the Laminin Beta 4 chain (LAMB4).

This allows the polymer act as a binding surface for adherent cells, including induced pluripotent stem cells (iPSCs), when used to coat materials such as plastic plates or microcarriers.



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LamininB4-Cytocaf

Coating protocol

For technical support:
info@marrabio.com

(NB: Use non-tissue culture treated plates to ensure effective coating.)

1. **Defrost** LamininB4-Cytocaf on the bench at room temperature or by placing the vial in a water bath at 37°C. The LamininB4-Cytocaf 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 LamininB4-Cytocaf to 10 µg/mL in sterile 1X PBS.

4. **Transfer** the solution to the centre of the well so that the final concentration is 1.5 µg/cm^2. See table for suggested volumes per plate/dish size.

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.

Plate	Coating solution (µL)
1586	4.5
384	12.5
96	50
48	175
24	300
12	550
6	1500

Dish (mm)	Coating solution (mL)
3.5	1.4
60	3.25
100	9
150	22

7. **Prepare cells** in cell culture medium to the required cell density.

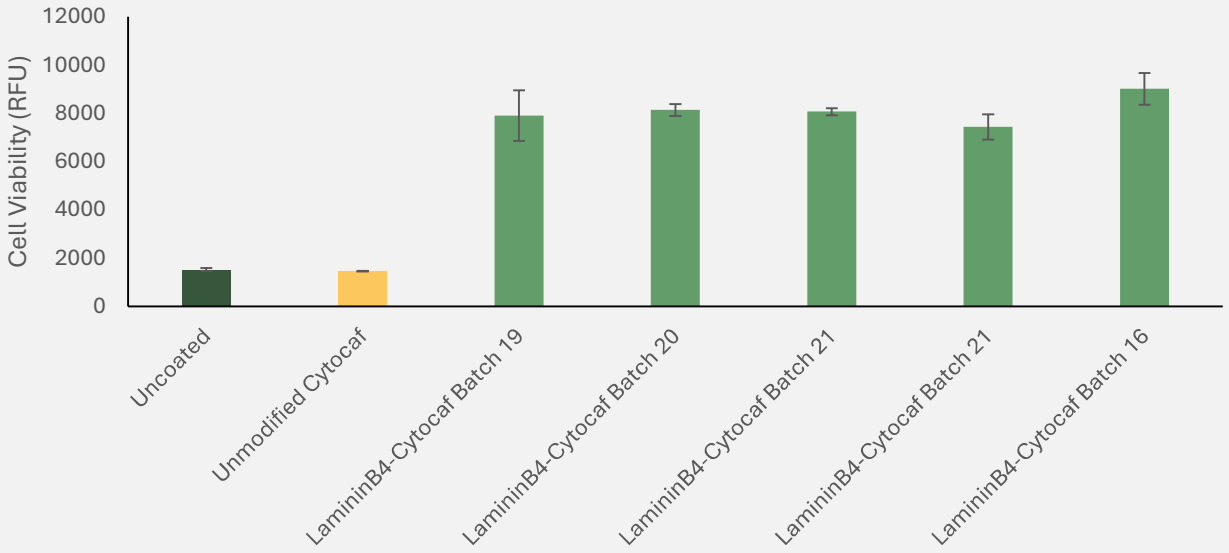
8. **Aspirate** the remaining LamininB4-Cytocaf solution from the wells.

9. **Seed cells** directly onto the coated surface at the required density and grow at 37°C in a CO_2 cell culture incubator.

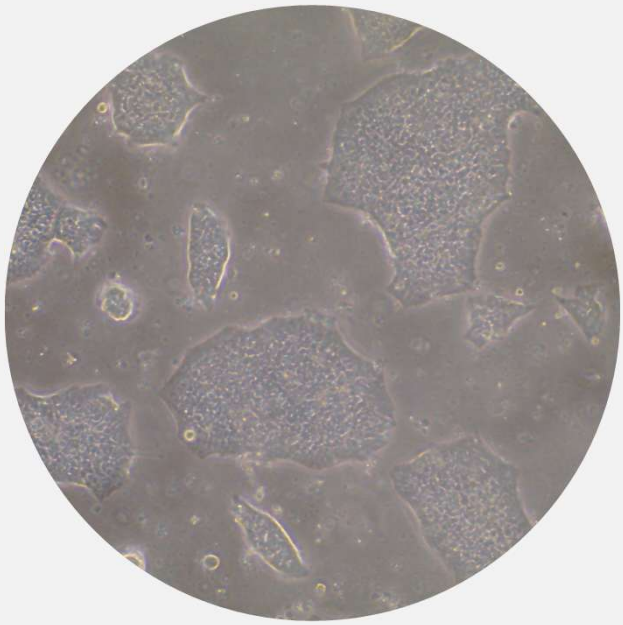
LamininB4-Cytocaf

Figure 1 : LamininB4-Cytocaf supports the growth of human iPS cells with high batch-to-batch consistency.

(A)



(B)



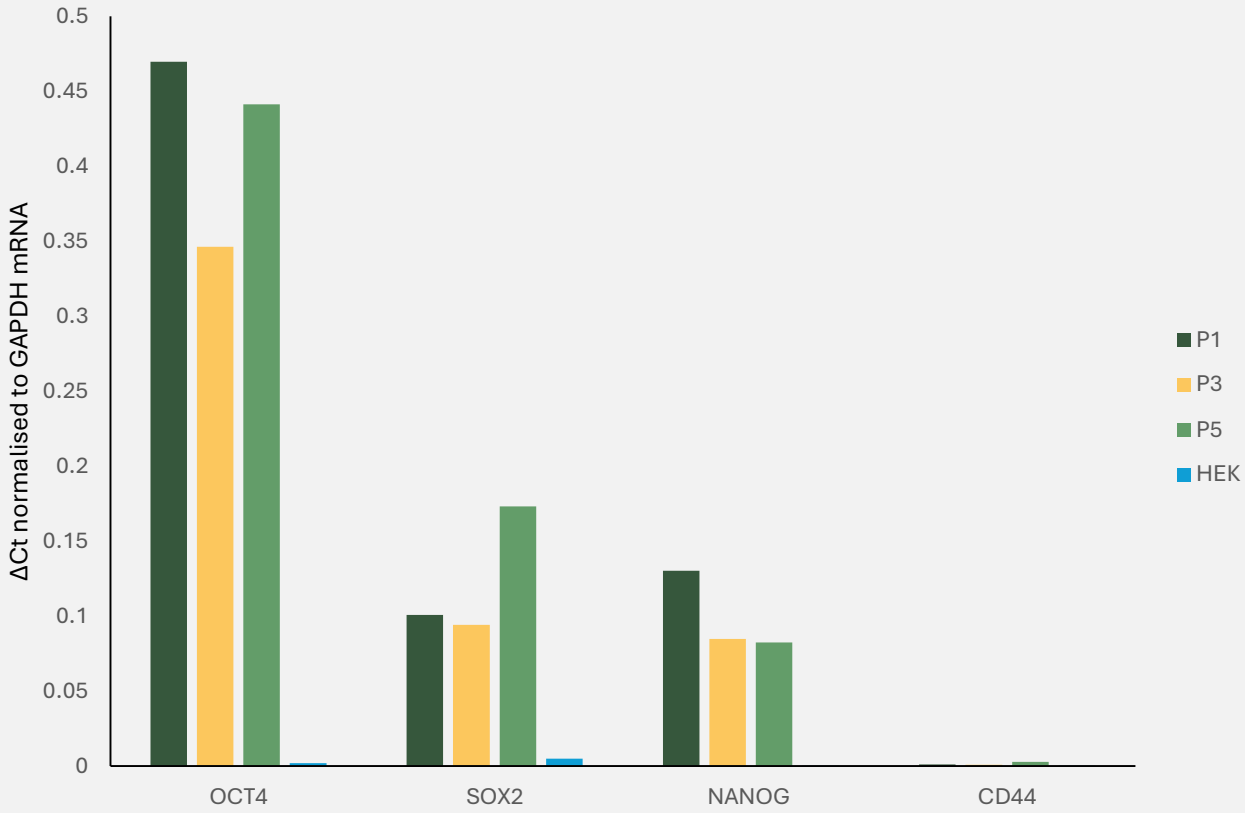
(A) Human iPSCs were seeded at 0.5 x 10^4 cells per well in wells of a non-tissue culture treated 96-well plastic plate that were either (i) uncoated, (ii) coated with unmodified Cytocaf or (iii) coated with different batches of LamininB4-Cytocaf, in E8 flex media supplemented with CloneR2. After 24 h the media was exchanged for fresh E8 flex media and cells were then grown for a further 24 hours. Cell viability was then measured using a PrestoBlue assay according to the manufacturer's instructions. Error bars represent SEM from three technical replicates.

(B) Human iPSCs were seeded onto LamininB4-Cytocaf coated non tissue culture treated 3.5 cm plates and grown for three days in E8 flex media, before imaging with a light microscope at 10X magnification.

LamininB4-Cytocaf

Figure 3 : Bioactivity

LamininB4-Cytocaf provides a bioactive adhesive surface for a range of cell types and is able to support the maintenance of phenotype in human iPSCs across multiple passages.

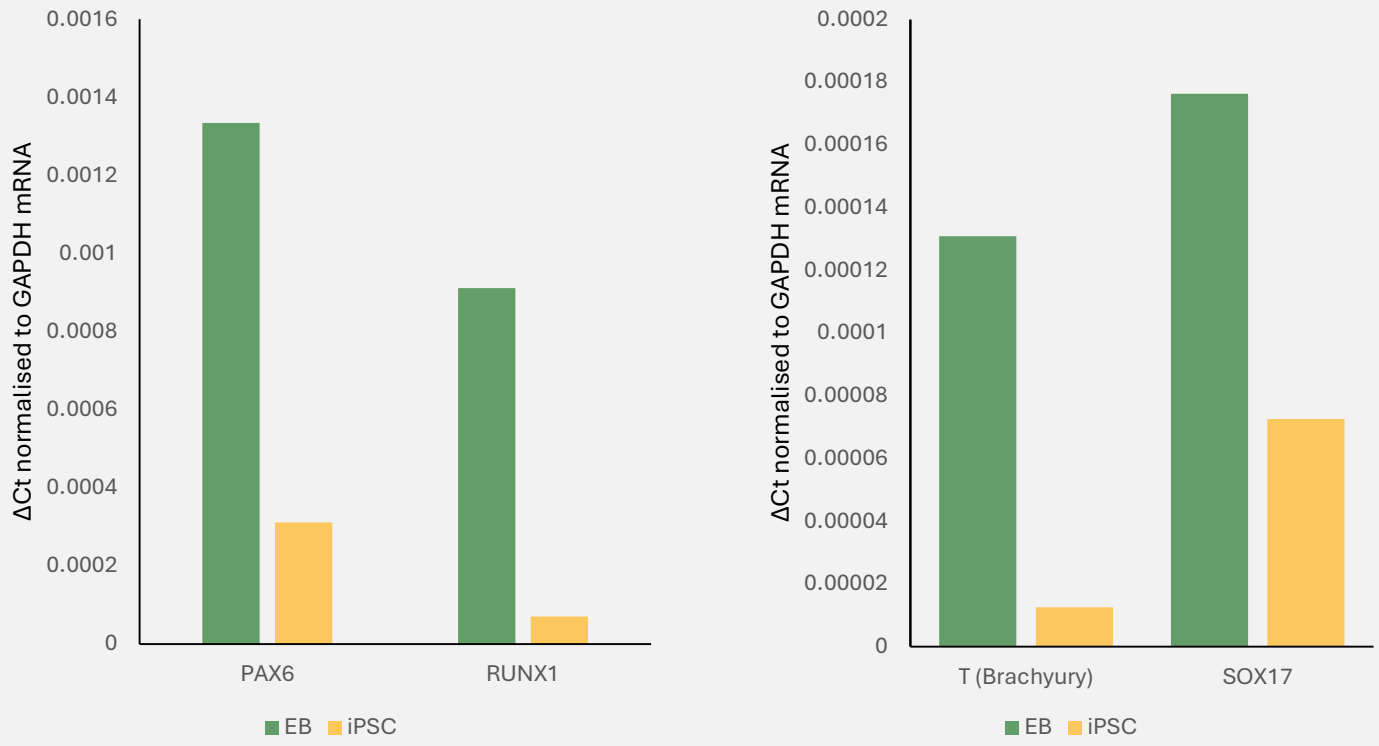


Human iPSCs were seeded at a 1 in 8 split ratio into 3.5 cm non-tissue culture treated dished pre-coated with LamininB4-Cytocaf and grown in E8 flex media supplemented with 10 µM rock inhibitor. After 24 hours, the media was changed and the cells were left to become 80% confluent with media changes performed every other day. Cells were subcultured in the same way. At passage 1, 3 and 5 cells were harvested for RNA analysis. RNA was harvested, reverse transcribed to cDNA and analysed using quantitative real-time PCR. OCT4, SOX2, and NANOG are well-known markers of iPSCs, and CD44 was used as the negative marker.

LamininB4-Cytocaf

Figure 4 : Bioactivity

iPSCs grown on LamininB4-Cytocaf retain their differentiation potential.

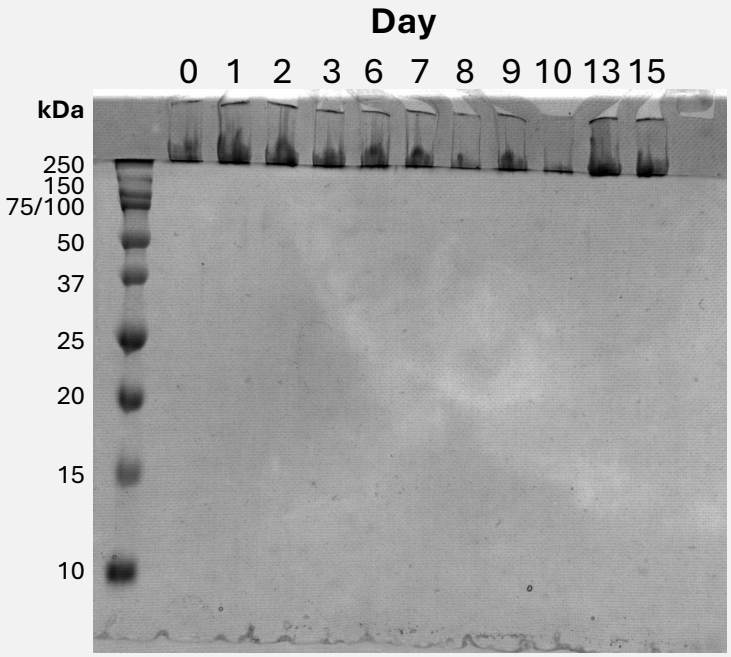


After 5 passages of human iPSCs on LamininB4-Cytocaf coated plates, cells were seeded at a split ratio of 1 in 5 into LowAdhere-Cytocaf (which is non-adhesive for iPSCs) coated 6 cm non-TC treated dish in E8 flex media supplemented with 4 mg/mL polyvinyl alcohol (PVA) and 10 µM ROCKi. After 24 hours the media was changed to E6 media with 4 mg/mL PVA. The same media exchange was performed every 2-3 days for 7 days before embryoid bodies were harvested for qPCR analysis.

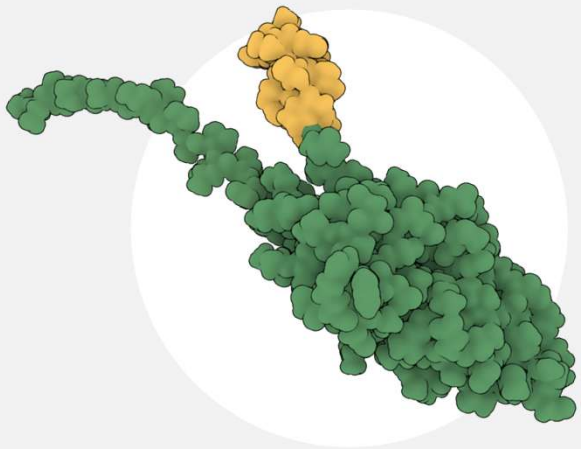
LamininB4-Cytocaf

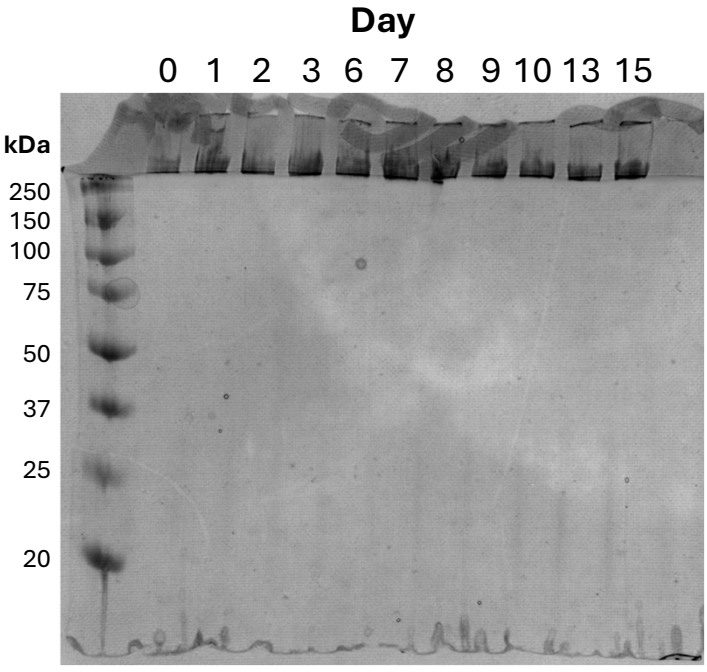
Figure 5: Stability

LamininB4-Cytocaf remains stable at room temperature with no signs of protein degradation even after 14 days of incubation at room temperature and 42°C.



Room Temperature





42°C

*(**A**) Samples of LamininB4-Cytocaf at 1 mg/mL were incubated at room temperature for the number of days shown, then analysed by SDS-PAGE.*

*(**B**) Samples of LamininB4-Cytocaf at 1 mg/mL were incubated at 42°C for the number of days shown, then analysed by SDS-PAGE.*

In both cases, the protein remains polymeric and so does not enter the resolving gel on account of its large size.