Confidential

Evaluation of Internationally Available Cell Culture Substrates for iPS Cell Expansion Using a Closed-System Automated Culture Platform

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CiRA Foundation

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Conflict of Interest (COI)

The author has **no conflicts** of interest to disclose.

What is the Best Extracellular Matrix (ECM) for Overseas Using Closed-System Automated Culture Platform?

Extracellular matrix (ECM)

Japan
iMatrix-511

For international use…
① **rhLaminin-521**
② **Synthemax**
③ **Vitronectin**

Pre-packed media





Human induced pluripotent stem cells (iPSCs)

CliniMACS Prodigy®, Miltenyi Biotec

Evaluation of Cell Attachment on Different ECM Coatings

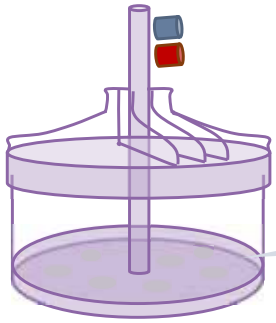
Preparation bag



ECM substrate	Diluent	Coating concentration
iMatrix-511	D-PBS(-)	250 μg/100 cm^2※
Laminin-521	D-PBS(+)	50 μg/100 cm^2
Synthemax	Sterilized water	500 μg/100 cm^2
Vitronectin ① (VTN-N)	D-PBS(-)	50 μg/100 cm^2
Vitronectin ② (Vitronectin XF)	Dilution Buffer	100 μg/100 cm^2

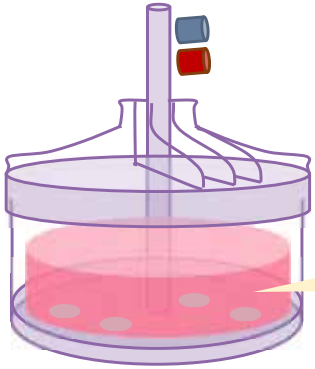
※5× recommended concentration (based on prior results)

Stored up to 1 month （4℃）
(Pre-prepared)
or
Freshly Prepared

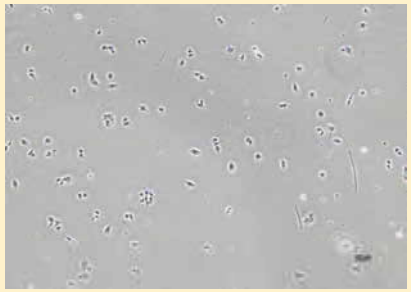


37℃, >12 h
ECM coating

Cell Seeding→ Culture→ Harvesting → Evaluation Workflow

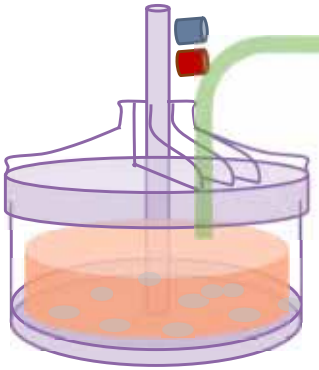


CFiS-S03 (iPSCs)
2×10^5 cells

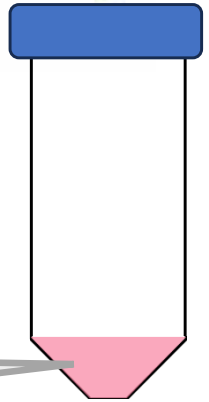


・Culture for 7 days (37℃, 5% CO_2)
・Change the medium every other day
(StemFit® **AK03N**：30mL)

★Colony Morphology → Microscopy



Cell harvesting



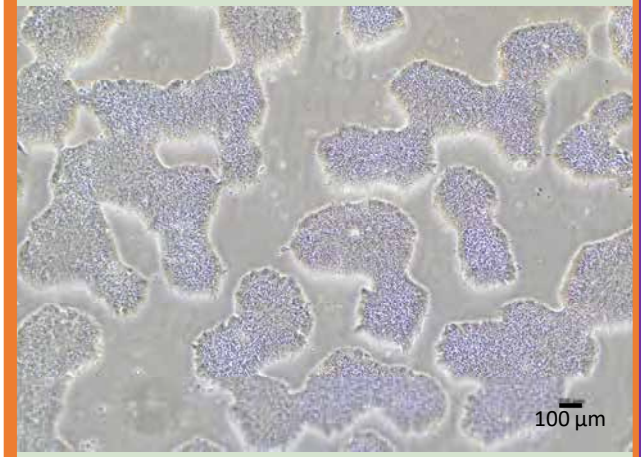
★ Proliferation → Cell count
★ Pluripotency → FCM

Result

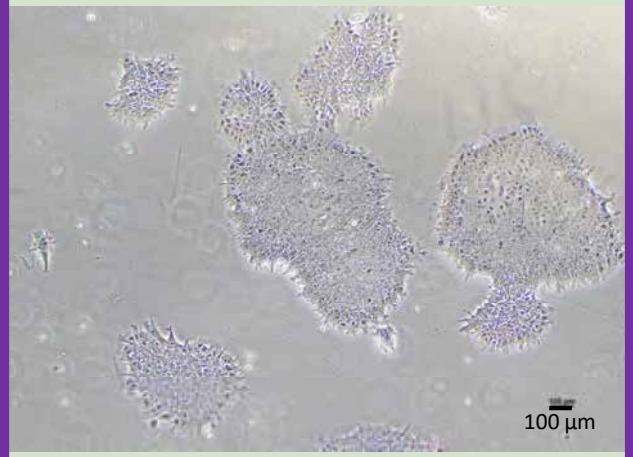
Representative Colony Morphology After 5 Days of Culture

Freshly Prepared

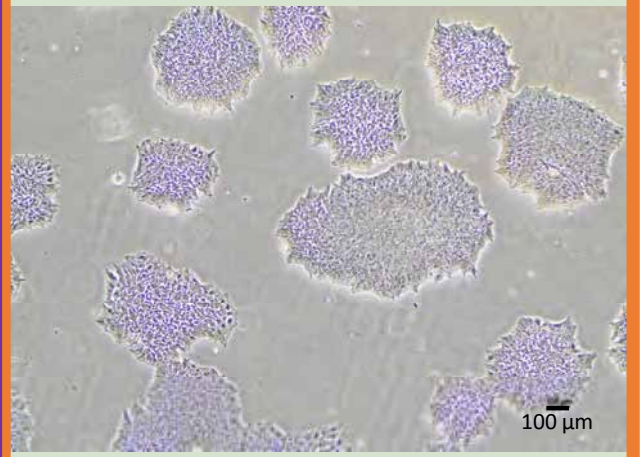
iMatrix-511



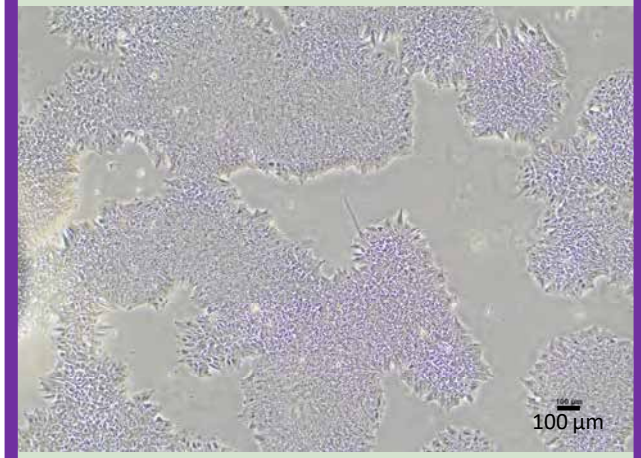
Laminin-521



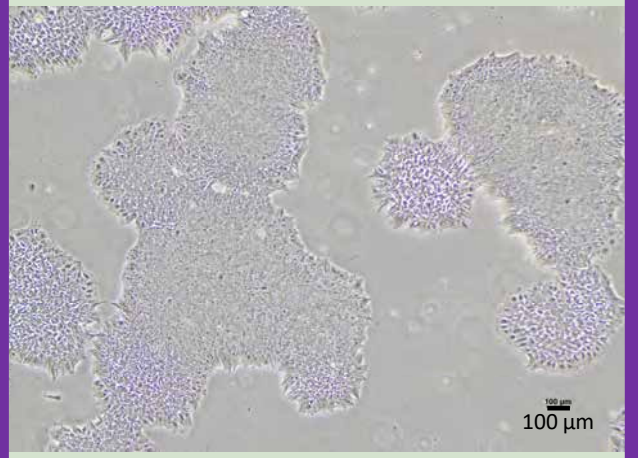
Synthemax



VTN-N



Vitronectin XF

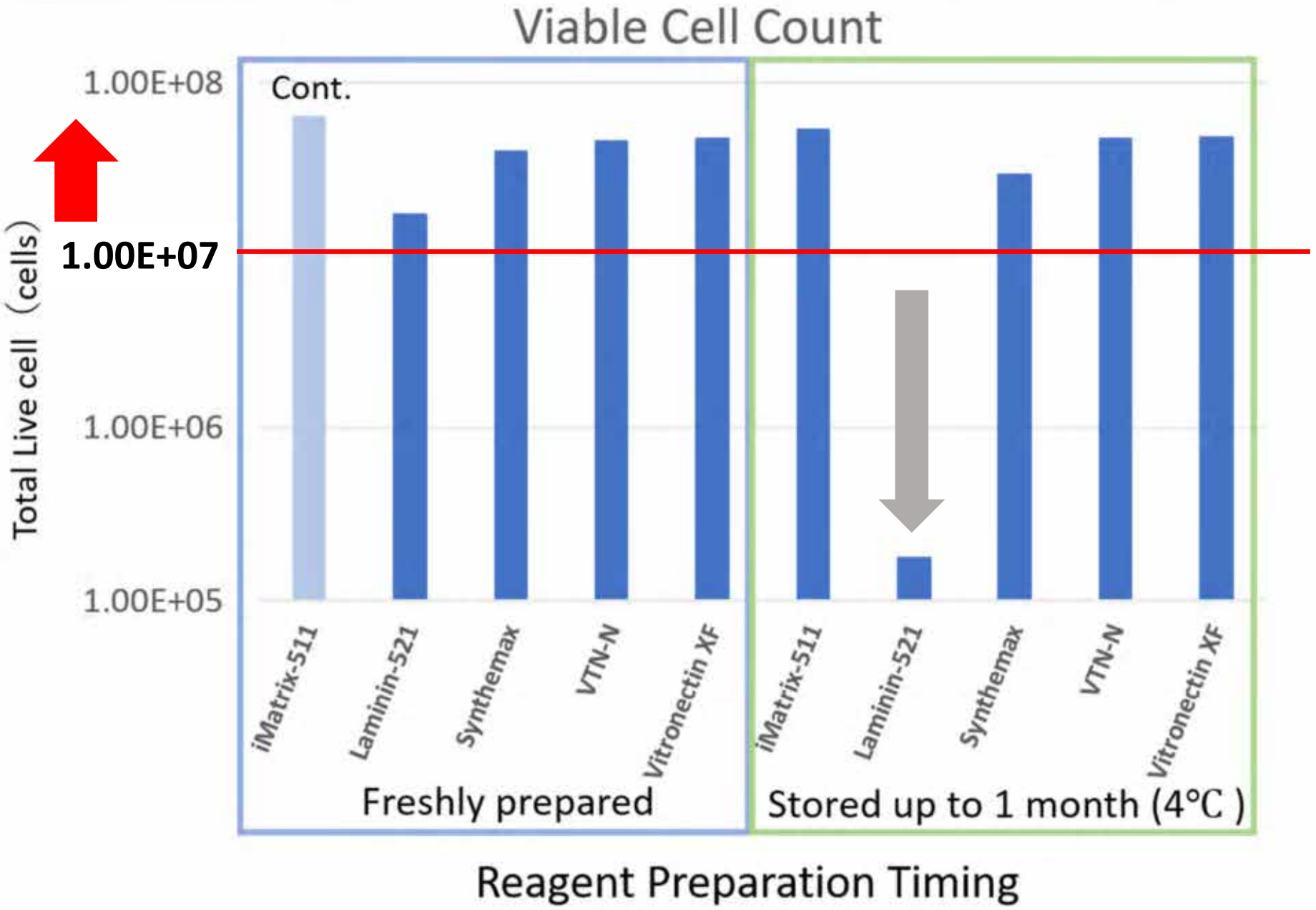


：Dome-shaped colonies

：Flat colonies

Result

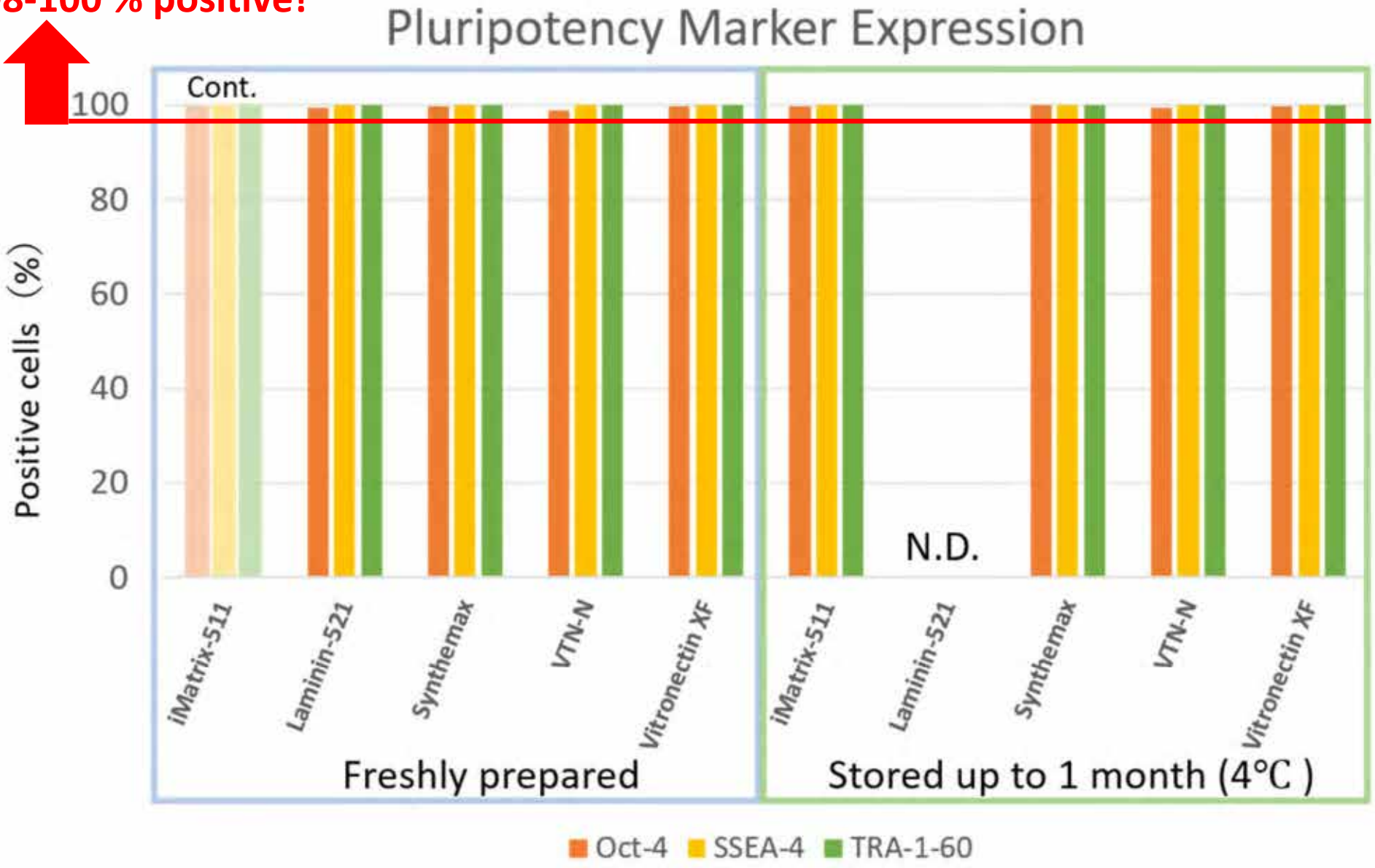
All Tested ECMs Supported iPSCs Attachment and Proliferation



Result

All Tested ECMs Showed Comparable Positivity for Pluripotency Markers

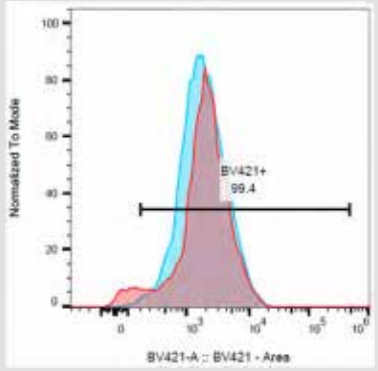
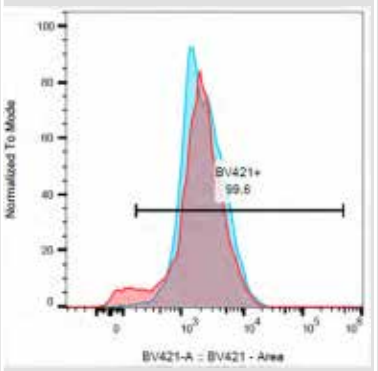
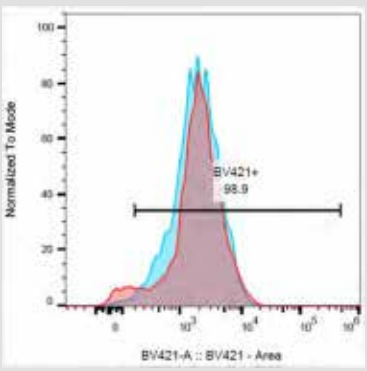
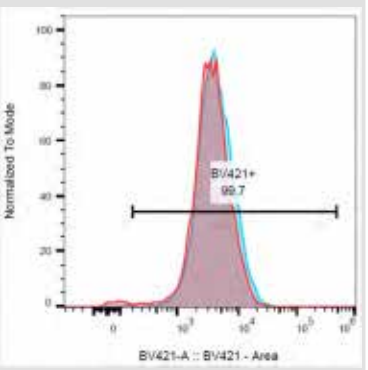
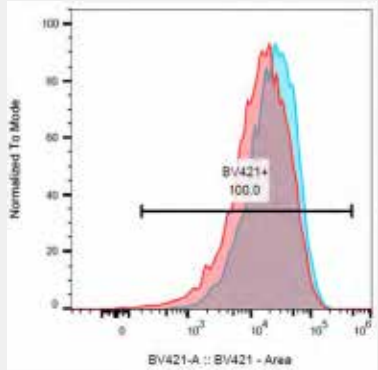
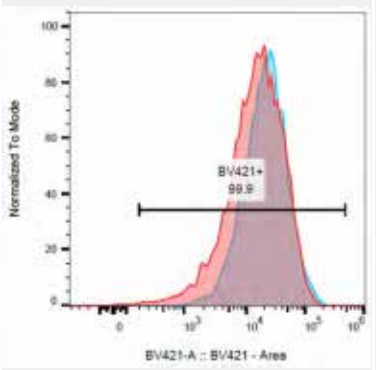
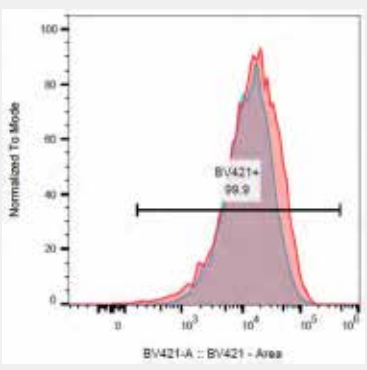
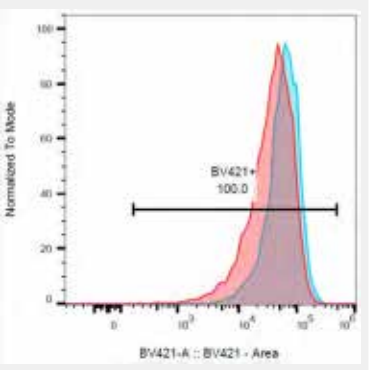
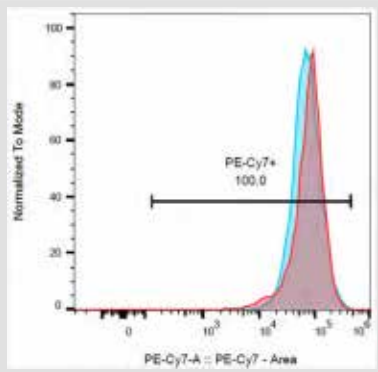
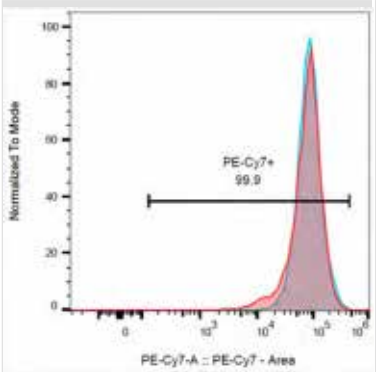
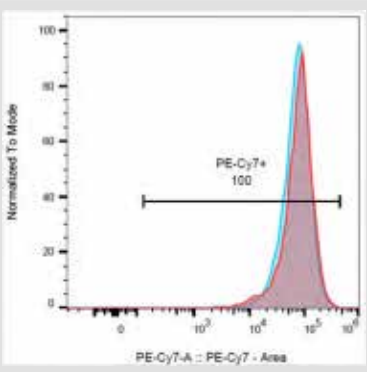
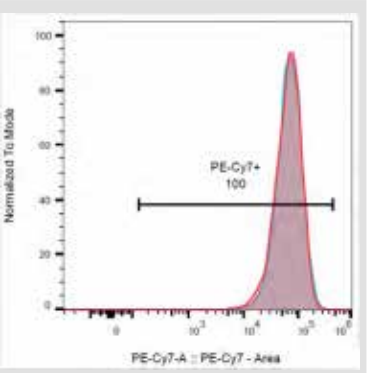
98-100 % positive!



Result

No Differences in Flow Cytometry Histogram Across ECMs

：Control (iMatrix-511, Freshly prepared)　　：ECMs **(Freshly prepared)**

	Laminin-521	Synthemax	VTN-N	Vitronectin XF
Oct3/4-BV421				
TRA-1-60-BV421				
SSEA4-PE-Cy7				

Conclusion

Synthemax Emerges as a Leading Candidate

	iMatrix-511	Laminin-521	Synthemax	VTN-N	Vitronectin XF
Cell Attachment & Proliferation	★★★	★★☆	★★★	★★★	★★★
Pluripotency Maintenance	★★★	★★★	★★★	★★★	★★★
Clinical-grade Availability	★★★	★★★	★★★	★★★	☆☆☆
Cost	★★☆	★☆☆	★★★	★★☆	—
Overall Assessment	★		★		

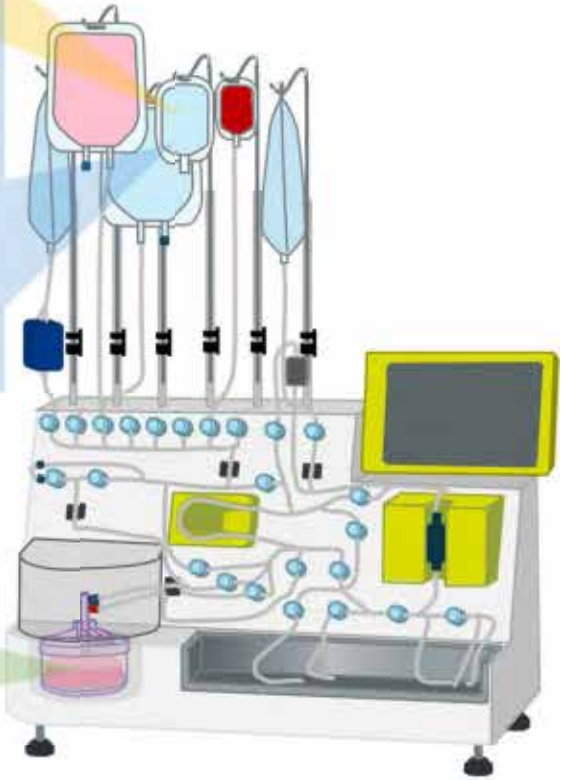
Future Work

Assessment of ECM concentration during bag-based preparation

Laminin-521

・Verify the reproducibility of the 1-month storage result (n = 1)

・Assess potential adsorption of Laminin-521 to the bag during storage at 4℃



Assess the suitability of the selected ECMs for iPSC generation from PBMCs

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