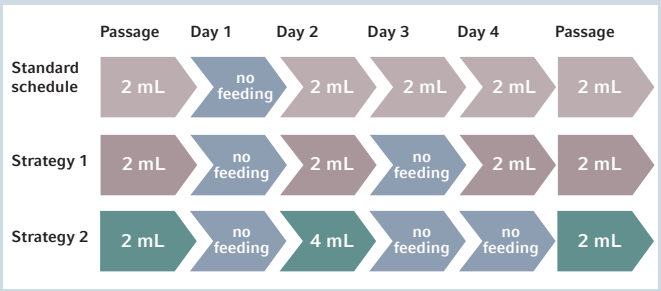
# Flexible and Xeno-Free hiPSC Culture System

Tired of spending precious lab time on coating vessels and changing medium every day?
Combine the Eppendorf CCCadvanced® FN1 motifs surface and Miltenyi StemMACS™ iPS-Brew XF to establish a flexible and xeno-free culture system for hiPSCs, which allows efficient transition, robust expansion and effective hiPSC recovery after cryopreservation, while maintaining cell pluripotency and trilineage differentiation potential.

## Ready-to-use growth surface

Coated with synthetic RGD-containing motifs, Eppendorf CCCadvanced® FN1 motifs surface combines experimental flexibility and performance robustness. Compatible with a broad range of cell dissociation reagents and growth media, this surface allows stable long-term expansion of various ECM-dependent stem and primary cells including hiPSCs in completely defined culture systems. Ready-to-use, this surface contributes to enhance experimental reproducibility while avoiding tedious coating.

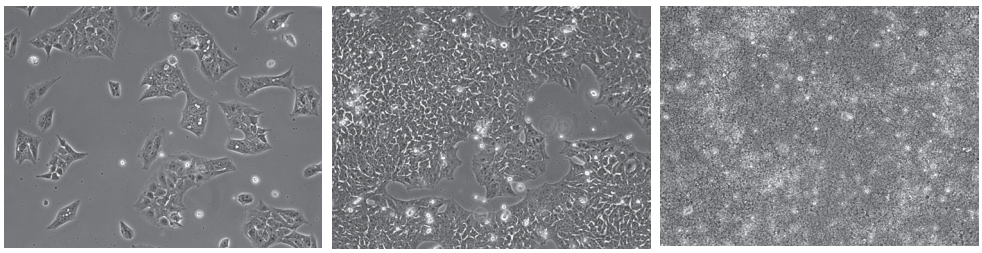
## Flexible feeding schedule




StemMACS™ iPS-Brew XF enables flexible feeding schedules, including the possibility of skipping one or even two feeding days, maintaining typical colony morphology, high expansion rate and pluripotency level over successive passages.

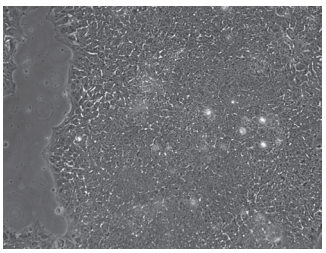
## Efficient transition and robust expansion

**High proliferation directly after transition on FN1 motifs**



D1 D3 D5

**Passage 5**



D3

hiPSCs (two independent cell lines, images shown for one clone) expanded for 5 passages in this completely defined culture system present their typical and stable morphology without spontaneous differentiation. After transition, hiPSC growth is consistent and robust with an efficient average population doubling time (+/- 25-26h, respectively for both hiPSC lines tested).

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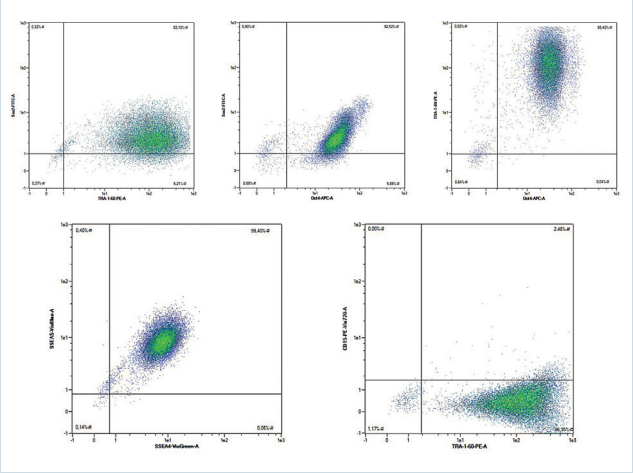
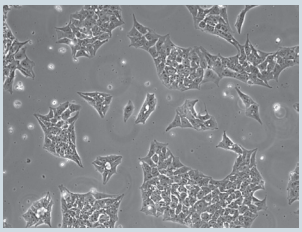
## Stable pluripotency markers expression



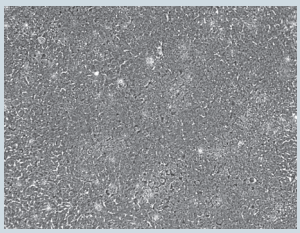
After 5 passages hiPSCs maintain a high expression level of pluripotent specific surface proteins, TRA-1-60 and SSEA4/5, and self-renewal-associated nuclear transcription factors, Oct3/4 and Sox2 shown by FACS analysis (two independent cell lines, data shown for one clone).

## Effective recovery after cryopreservation

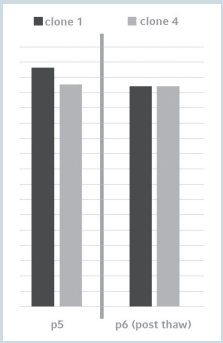
### Efficient cell growth post-thawing



D2 post-thawing



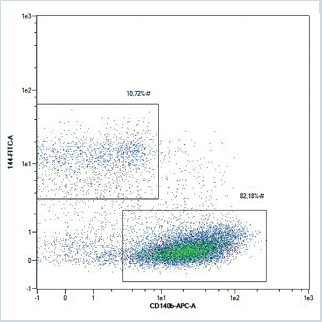
D4 post-thawing



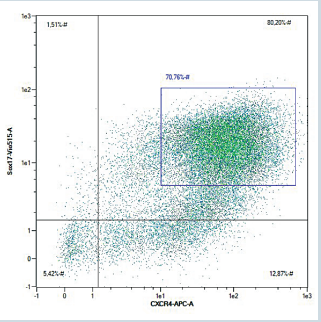

### Stable doubling time post-thawing

StemMACS™ Cryo-Brew allows a high recovery rate of hiPSCs on FN1 motifs surface with StemMACS iPS-Brew. FACS analysis confirms the expression of key pluripotency markers after thawing (data not shown).

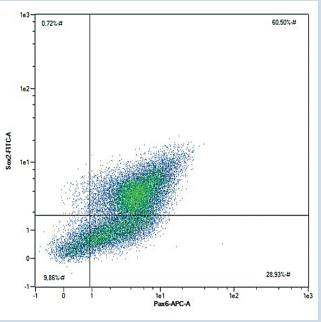
## Maintenance of trilineage differentiation potential



Mesoderm



Endoderm



Ectoderm

After 6 successive passages, hiPSCs (two independent cell lines, data shown for one clone) have been differentiated using StemMACS Trilineage differentiation kit. FACS analysis with key lineage-specific markers performed on differentiated cells confirms the preservation of a functional trilineage differentiation potential.

## Conclusions

> The combined use of ready-to-use Eppendorf CCCadvanced FN1 motifs surface and Miltenyi StemMACS iPS Brew XF culture medium improves the flexibility of the routine hiPSC workflow while ensuring a robust and stable stem cell expansion in a xeno-free culture system.

> Being ready-to-use, the FN1 motifs surface allows to reduce significantly labor time and effort while offering improved lot-to-lot consistency and more reliable performances in comparison with self-coating solutions.

> The StemMACS iPS Brew XF culture medium ensures a rapid and smooth culture system transition from undefined biological matrix to the synthetic FN1 motifs surface associated with a consistent and robust growth rate and the maintenance of key pluripotent stem cell-specific features including the trilineage differentiation potential.

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