Miltenyi Biotec

StemMACS™ iPS-Brew XF – FAQs

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Which matrices can I use with StemMACS™ iPS-Brew XF?

StemMACS iPS-Brew XF works great with any of the following matrices: Matrigel®, Geltrex®, Laminin-521, Vitronectin, CTS™ CELLstart™ Substrate, and iMatrix-511. At Miltenyi Biotec, we prefer to use Laminin-521 as it is a xeno-free substrate with clinical relevance and provides superior attachment properties.

Which passaging method do you recommend?

All common passaging techniques will work. Our customers use StemMACS iPS-Brew XF in combination with traditional colony cutting, clump-splitting as well as enzymatic single cell passaging techniques – it's your choice!
At Miltenyi Biotec, we prefer to use StemMACS Passaging Solution XF, because it is a very quick and easy method: Remove the old culture media and wash once with PBS. Then, simply add StemMACS Passaging Solution XF for 4 minutes and the colony edges will start to lift off. After removing the solution, use fresh media to dissociate your colonies into clumps and transfer to a new dish.
To enhance attachment on new PSC lines, we recommend including a ROCK inhibitor such as StemMACS Y27632 (10 µM final concentration) or StemMACS Thiazovivin (2 µM final concentration) for the plating. The ROCK inhibitor can be removed with the next media change.

What is the best way to transition my culture to StemMACS iPS-Brew XF?

Simply seeding your cells in StemMACS iPS-Brew XF after splitting will work for many cell lines (supplement with a ROCK inhibitor as needed to facilitate attachment). However, the more gentle way is a gradual transition to the new culture media. If your cell line has been in another media environment for a long time or is generally sensitive to change, exposing it to a new media environment and asking for re-attachment at the same time may be too much.
To achieve a gradual transition, expose your culture to StemMACS iPS-Brew XF while colonies are still attached: 1–2 days before the split, replace media with a 50:50 mixture of StemMACS iPS-Brew XF and the previous media. After the split, use StemMACS iPS-Brew XF only.

I see spontaneous differentiation upon switching to StemMACS iPS-Brew XF. What can I do?

Pluripotent stem cells grown in StemMACS iPS-Brew XF have usually high expansion rates. If you have been using a media that supports lower proliferation rates, you may need to adapt your splitting ratio in order to avoid overgrowth. During the first passages, closely watch the confluency of your culture and – if necessary – remove differentiated cells either manually or using the Anti-TRA-1-60 MicroBeads. You should not see spontaneous differentiation beyond passage 3.
Note: The presence of ROCK inhibitor can induce a fibroblast-like morphology, especially after single cell splitting or with small colony pieces. This is not a sign of differentiation and will disappear at larger colony size/ withdrawal of the inhibitor.

Colonies do not attach completely. What can I do?

Be patient: Suboptimal attachment is one of the most common problems during the transition phase. Make sure you are using a ROCK inhibitor (StemMACS Y27632 or StemMACS Thiazovivin). As long as colonies don't detach completely, let them grow in size and passage the culture 2–3 times in StemMACS iPS-Brew. You should not see this anymore beyond passage 5.
If you are using single cell splitting, seed at least 120,000 cells per 6-well. Use a matrix that supports strong attachment (e.g. laminin-521).

Do I have to feed my culture every day?

No. StemMACS iPS-Brew XF supports flexible feeding schedules, including the possibility for every-other-day feeding or even week-end-free culture. For examples of different 5-day splitting schemes, download this **Application Note**.

Note: We recommend sticking to the traditional daily feeding rhythm during the initial transition to StemMACS iPS-Brew XF from other culture media. Also, for ES/iPS cell lines that are prone to spontaneous differentiation, we recommend to continue with daily feeding.

How is StemMACS iPS-Brew XF different from other PSC culture media?

StemMACS iPS-Brew XF is based on a carefully optimized, proprietary basal media formulation. This basal media has been developed at Miltenyi Biotec and is specifically tailored to the metabolic needs of human pluripotent stem cells.

In addition, StemMACS iPS-Brew XF includes all growth factors necessary for maintenance of highly pluripotent cultures. All growth factors are manufactured in Miltenyi Biotec's own production facilities in Germany and assayed for biological activity. Dosing based on unit activity (U/mL) is one of the key factors for the extremely high lot-to-lot consistency of StemMACS iPS-Brew XF.

The number of components in StemMACS iPS-Brew is significantly reduced compared to complex media like mTeSR1 and is sufficiently defined to allow the production of a corresponding GMP-grade.

Can StemMACS iPS-Brew XF be used for suspension cultures?

Yes, StemMACS iPS-Brew XF has been used for suspension culture of pluripotent stem cells. The specific culture protocol must be established for the respective cell line and type of culture vessel individually.

Can cells grown in StemMACS iPS-Brew XF differentiate into all cell types?

Pluripotent stem cells grown in StemMACS iPS-Brew XF show normal teratoma formation with differentiation into derivatives of all three germ layers. Likewise, cells maintained in StemMACS iPS-Brew XF have been used for directed differentiation into various cell types without any indication of a differentiation bias.

When will StemMACS iPS-Brew become available in GMP-grade?

The corresponding GMP-grade will be available in 2016.

Which QC assays do you perform for StemMACS iPS-Brew XF?

Each lot of StemMACS iPS-Brew XF is tested for 5 consecutive passages for its performance in Matrigel®-based iPS cell culture. Culture quality is assessed by morphology, growth rate and surface phenotype (TRA-1-60, SSEA-4, SSEA-3, SSEA-1). In addition, StemMACS iPS-Brew XF is tested for low endotoxin and absence of mycoplasma.

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