



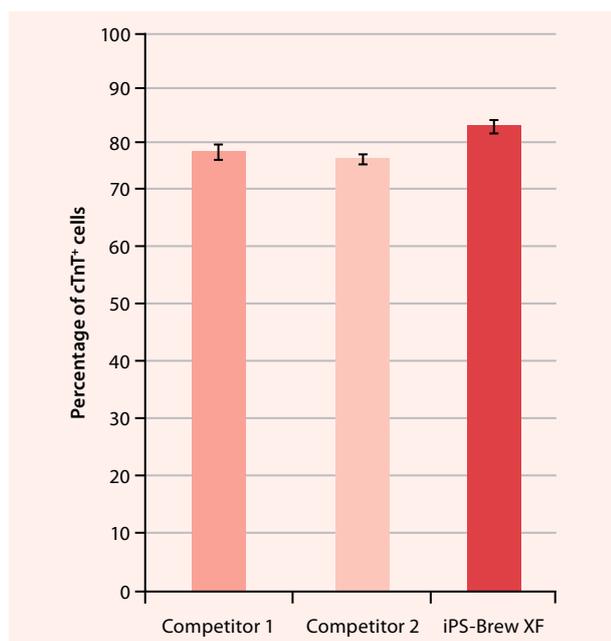
## Frequently asked questions

StemMACS™ CardioDiff Kit XF, human

StemMACS™ Cardiac Cultivation Medium XF, human

### Can I use the StemMACS™ CardioDiff Kit XF if my hPSCs were not cultivated in StemMACS iPS-Brew XF?

Yes, the StemMACS CardioDiff Kit supports cardiac differentiation of human pluripotent stem cell (hPSCs) that were cultured in StemMACS iPS-Brew XF, but also in other commercially available stem cell media (figure 1). However, given that StemMACS iPS-Brew XF has demonstrated robust and stable performance, and that the media is available in MACS GMP grade to enable a seamless translation from research to clinical applications, we strongly recommend using it.



**Figure 1:** Percentage of cells positive for cardiac troponin T (cTnT). StemMACS CardioDiff Kit XF supports cardiac differentiation of hPSCs that were cultured in StemMACS iPS-Brew XF, and other commercially available pluripotent stem cell media.

### What is the best hPSC quantity to start the differentiation?

We strongly recommend performing a pre-trial titration when starting the differentiation with a new hPSC line. Some lines may require higher starting cell number than others. Suggested cell numbers for a titration trial are 125,000 cells/cm<sup>2</sup>, 250,000 cells/cm<sup>2</sup>, 300,000 cells/cm<sup>2</sup>, and 400,000 cells/cm<sup>2</sup>.

### When can I see contracting cardiomyocytes in the culture?

The first contracting cardiomyocytes can be observed after day 6, although this is strictly dependent on the hPSC line and some lines may require a little longer.

### Can I use the StemMACS CardioDiff Kit XF to differentiate ESCs?

Yes, the StemMACS Cardio Diff Kit supports the cardiomyocyte differentiation of several different hPSC lines, either induced PSCs (iPSCs) or embryonic stem cells (ESCs) with good efficiency rates.

### How long can I culture the cells?

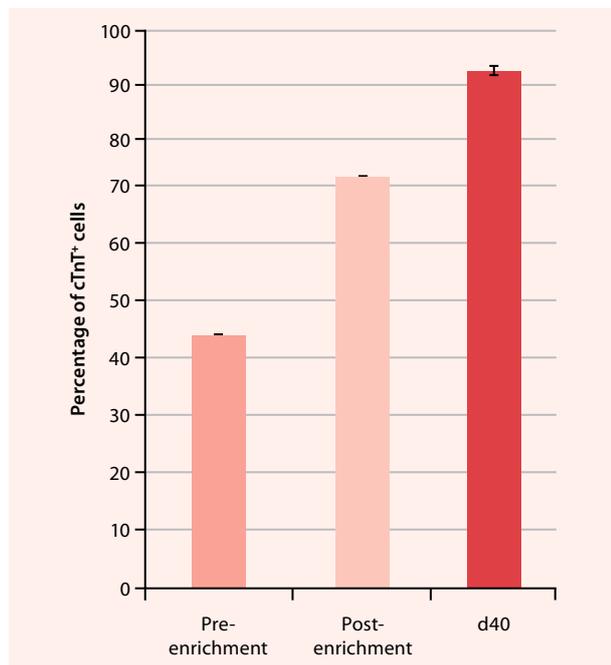
Cardiomyocytes can be maintained in culture for more than 30 days, if fed every two days with StemMACS Cardiac Cultivation Medium XF. For long term culture we recommend monitoring the cells and transferring them into new culture plates as soon as early sign of detachment from the border is visible.

### Can I freeze cardiomyocytes obtained with StemMACS CardioDiff Kit XF?

Yes, cardiomyocytes obtained with StemMACS CardioDiff Kit XF and cultured in StemMACS Cardiac Cultivation Medium XF can be frozen for long term storage in StemMACS Cryo-Brew. The use of StemMACS Cryo-Brew ensures high thawing efficiency and cell viability, and also allows for rapid culture re-initiation. Additionally, the formulation of StemMACS Cryo-Brew allows cryopreservation under xeno- and serum-free conditions.

**I am working with a hPSC line that fails many differentiation protocols and results in low differentiation efficiencies, can I use the StemMACS™ CardioDiff Kit XF?**

The StemMACS CardioDiff Kit XF supports cardiomyocyte differentiation of several different hPSC lines with good efficiency rates. However, some lines are known to not respond well to many induction protocols. In these cases, we recommend performing the differentiation with the StemMACS CardioDiff Kit XF and after day 10, isolate the cardiomyocytes with the PSC-Derived Cardiomyocyte Isolation Kit, human to enrich the cardiomyocyte culture. The kit provides two different strategies for cardiomyocyte enrichment, which can be applied depending on the initial differentiation efficiency. Moreover, prolonged culturing with StemMACS Cardiac Cultivation Medium XF after enrichment, further increases the purity of the cardiomyocyte population (figure 2).



**Figure 2:** PSC-derived cardiomyocytes culture enrichment of a low efficiency differentiation. The percentage of cells positive for cTnT was measured before enrichment (pre-enrichment), after cardiomyocyte isolation with the PSC-Derived Cardiomyocyte Isolation Kit, human (pre-enrichment) and after the enriched fraction was further cultivated in StemMACS Cardiac Cultivation Medium XF until day 40.

**Relevant products**

Product	Order no.
Anti- $\alpha$ -Actinin (Sarcomeric)-FITC, human, mouse, rat	130-124-060
Anti-Cardiac Troponin T-FITC, human, mouse, rat	130-119-674
Anti-MLC2a-APC, human, mouse, rat	130-118-674
Anti-MLC2v-PE, human, mouse, rat	130-119-680
Anti-Myosin Heavy Chain-APC, human, mouse, rat	130-122-924
Multi Tissue Dissociation Kit 3	130-110-204
PSC-Derived Cardiomyocyte Isolation Kit, human	130-110-188
StemMACS Cardiac Cultivation Medium XF, human	130-125-287
StemMACS CardioDiff Kit XF, human	130-125-289
StemMACS Cryo-Brew	130-109-558
StemMACS iPS-Brew XF, human	130-104-368



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