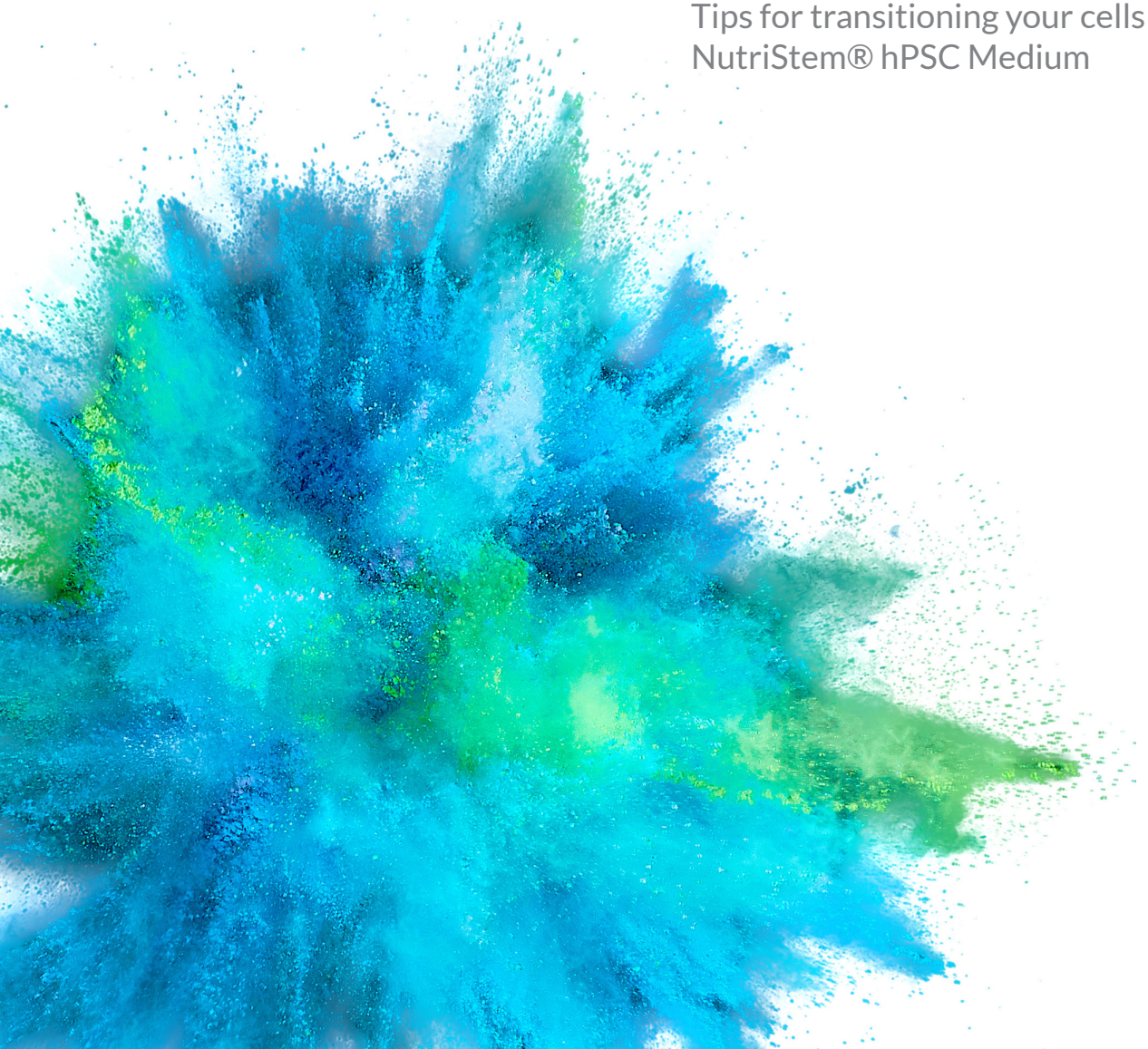
BI

GETTING STARTED

Tips for transitioning your cells to NutriStem® hPSC Medium



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Ultimate Control

NutriStem® hPSC Medium is a powerful low-bFGF medium which works across research and clinical workflows giving you ultimate control over what goes into your cultures.

Flexible and compatible

Flexible feeding schedule (including weekend-free options). Ability to choose the matrix and passaging reagent that best suits your specific applications. Optional enhancement with additional bFGF or HSA for various culture needs.

cGMP formulation

Produced under cGMP. Eliminates the need to switch media during clinical translation.

Enhances clean cell growth

The very first defined, xeno-free, serum-free media for hPSCs. Does not contain excessive amounts of growth factors or animal components which may promote differentiation.

Low amounts of bFGF

Compared to most commercially available high-bFGF media, NutriStem® hPSC Medium contains low amounts of bFGF which enables control over what goes into your cultures.

Only essential ingredients

Low-protein formulation contains only the most essential components required for maintenance of hES and hiPS cells, providing a simplified medium while maintaining the cells' full differentiation potential.

FDA Drug Master File

The first xeno-free, serum-free media to receive an FDA DMF on record. No licensing restrictions.

Customizable

Several packaging and volume fill options available including bags for bioprocessing. Fully growth factor-free version available.

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Tips for transitioning your cells to NutriStem® hPSC Medium

1 FGF-withdrawal

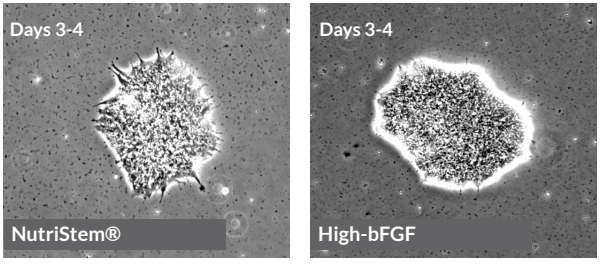
Morphology changes are normal and expected

Most commercially available media for human pluripotent stem cell (hPSC) maintenance contain high amounts of bFGF (up to 100 ng/mL) compared to BI's NutriStem® hPSC Medium, a low-bFGF medium (<10 ng/mL). When transitioning cells from a high-bFGF containing media to NutriStem® hPSC Medium, users should be aware that differences in morphology and growth rate are common. These differences, which can last for the first 2-3 passages, can largely be attributed to the hPSCs going through what can be described as a period of "FGF-withdrawal" as they become less and less dependent upon high amounts of bFGF over time. Consider gradually decreasing bFGF over time by initially supplementing NutriStem® hPSC Medium with additional bFGF (i.e. adding a 20 ng/mL boost) or by initially mixing NutriStem® hPSC Medium with the high-bFGF medium and gradually stepping down (75:25, 50:50, 25:75) to fully convert to 100% NutriStem® hPSC Medium.

2 Spikey edges

Morphology may appear flat, large, and spikey

When transitioning your cells to NutriStem® hPSC Medium, cells may appear larger and flatter compared to cells cultured in high-bFGF media - this is normal. Edges of colonies may be less compact than cells in the center of colonies, occasionally with elongated protrusions or "spikey" edges.

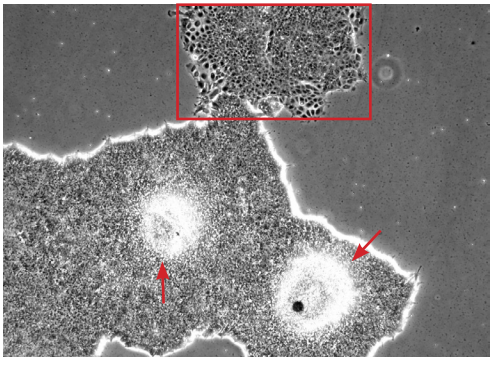


When small, low-density colonies are first established after a passage, the cells within the colony may appear larger than expected with low-bFGF cultures but are simply in a more delicate state. Without prior knowledge, this subtle morphology change can be mistaken for what may be spontaneous differentiation but is normal when transitioning to NutriStem® hPSC Medium.

3 Keep feeding

Slow and steady wins the race

The key to successfully transitioning cells to NutriStem® hPSC is patience – keep feeding the cultures daily, even if they look a bit "different" at first. Spontaneous differentiation in hPSC cultures transitioned to NutriStem® hPSC from high-bFGF media should be minimal. However, if small areas of differentiation appear early during the transition and adaptation phase, they should be removed immediately via scraping, picking, and/or aspiration.



4 Let's ROCK

Improve attachment with a ROCK inhibitor

ROCK inhibitor supplementation is not necessary when passaging hPSCs that are fully adapted to NutriStem® hPSC Medium. However, higher than average cell death may occur when initially passaging from high-bFGF media to NutriStem® hPSC Medium, or when thawing hPSCs directly into NutriStem® hPSC from vials cryopreserved using high-bFGF media. In these cases, ROCK inhibitor supplementation during the passage or thaw event may improve initial cell attachment and recovery upon thaw. We do not recommend supplementing the medium with ROCK inhibitor after the first day.

5 Adding a boost

Adding extra bFGF for sensitive cells

Some cell lines and applications do seem to need more convincing when it comes to maintaining their pluripotency, but having a minimal formulation provides wiggle room to accommodate these needs. NutriStem® hPSC Medium has been designed with a low bFGF concentration, and may be spiked with bFGF to facilitate adaptation or faster cell growth. For example, when reprogramming, labs will often increase bFGF levels by adding 20 ng/mL until iPSCs are established and then bring the culture back down to a lower bFGF level for routine culture. For 3D applications, higher amounts of bFGF may facilitate successful cultures in bioreactors.

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