TeSR™-AOF

Reduce Risk and Variability in Human Pluripotent Stem Cell Cultures

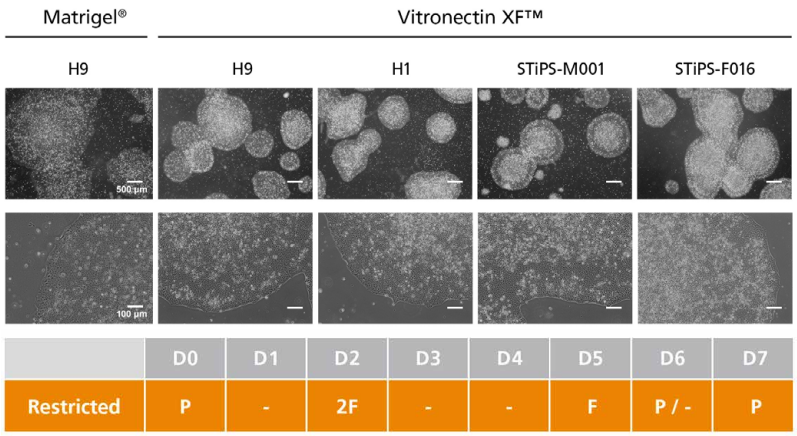
Prepare for a seamless transition to the clinic with TeSR™-AOF, the new animal origin-free (AOF) human pluripotent stem cell (hPSC) maintenance medium. Since it contains no animal-derived raw materials, TeSR™-AOF helps minimize risk in your cell therapy development—no matter what stage your research is at. Compared to low-protein formulations, TeSR™-AOF consistently supports cell growth across cell lines and allows for restricted feeding schedules, enabling you to get more of the cells you need while minimizing the time spent maintaining them. Read on to learn more about the features that make TeSR™-AOF a perfect fit for developing hPSC-based cell and gene therapies.

FEATURE 1 Animal Origin-Free

Ease the path to clinical applications with a medium that is viral-safe by design, with straightforward traceability documentation available for regulatory approval. TeSR™-AOF is manufactured without using raw materials (or the components used to make them) derived from an animal (including human) tissue, cells, or body fluid. This ensures animal origin-free traceability of raw materials to the secondary level of manufacturing, for an increased safety profile. For more information, see our detailed definitions at www.stemcell.com/media-definitions.

FEATURE 2 Promotes Consistent Cell Growth

Reliably generate more of the cells you need for your clinical applications. TeSR™-AOF supports high-quality colony morphology, robust attachment, and cell expansion across a wide range of cell lines.



	D0	D1	D2	D3	D4	D5	D6	D7
Restricted	P	-	2F	-	-	F	P / -	P

P = passage, F = feed, 2F = feed with 2X volume

Figure 1. hPSCs Cultured in TeSR™-AOF with Restricted Feeding Show Classic hPSC Colony Morphology

hPSC lines maintained in TeSR™-AOF and on Matrigel® or Vitronectin XF™ were passaged as aggregates with ReLeSR™ passaging reagent every 6 - 7 days. hPSCs maintained in TeSR™-AOF for greater than 10 passages exhibit hPSC-like morphology, by forming densely packed, round colonies with smooth edges. Homogeneous cell morphology characteristic of hPSCs is observed, including large nucleoli and scant cytoplasm. Unless otherwise noted, all data for TeSR™-AOF shown here and in subsequent figures were obtained using a restricted feeding schedule (bottom panel).

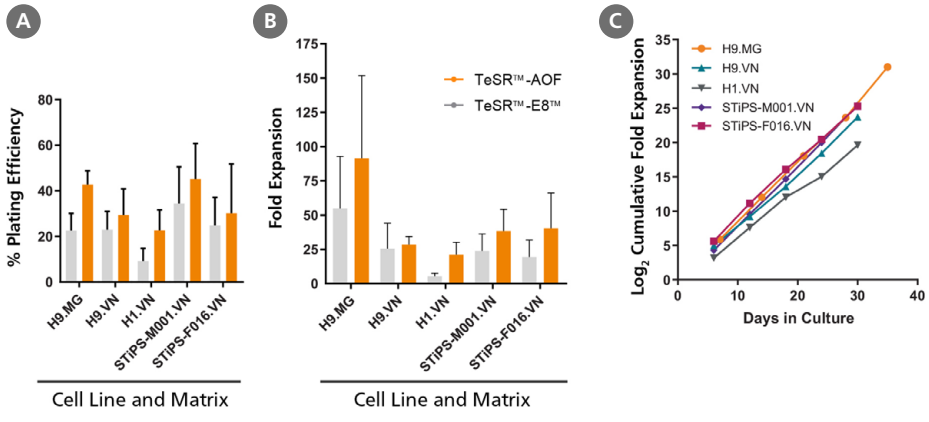


Figure 2. hPSCs Maintained in TeSR™-AOF Have Improved Attachment and Higher Overall Expansion Compared to Low-Protein Medium

(A) hPSCs cultured in TeSR™-AOF demonstrate a higher plating efficiency compared to hPSCs maintained in low-protein medium (TeSR™-E8™). Plating efficiency is calculated by seeding a known number of aggregates and comparing to the number of established colonies on day 7. (B) hPSCs maintained in TeSR™-AOF exhibit a higher average fold expansion per passage compared to TeSR™-E8™. (C) hPSCs cultured in TeSR™-AOF demonstrate consistent expansion and minimal variability across all cell lines assessed. Cumulative fold expansion was measured from passage 1 to 5. Data represented as mean plating efficiency or fold expansion across 10 passages ± SD. MG = Matrigel®; VN = Vitronectin XF™.

FEATURE 3 Stabilized Components

Stabilized components, including FGF2, support high cell quality while allowing for restricted feeding schedules. This means that, compared to following a daily feeding schedule, you can save time and media while still obtaining large numbers of high-quality cells.

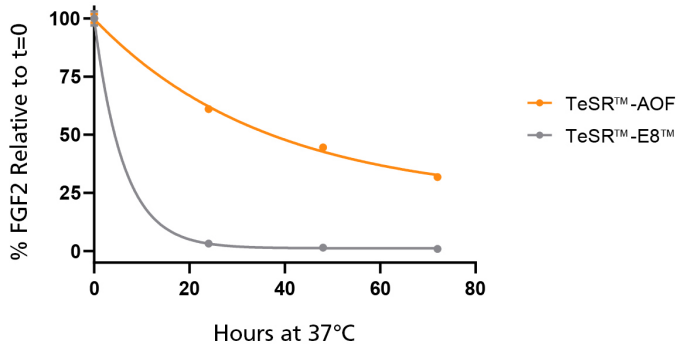


Figure 3. Native FGF Levels Are Stabilized at 37°C in TeSR™-AOF, Allowing Restricted Feeding Schedules

TeSR™-AOF and TeSR™-E8™ were incubated at 37°C for 24, 48, and 72 hours. FGF2 levels were measured by Meso Scale Discovery (MSD) immunoassay; data was normalized to t = 0 levels for TeSR™-E8™ and TeSR™-AOF, respectively. FGF2 levels in TeSR™-AOF decline much more slowly than in TeSR™-E8™, with 36.7 ± 5.61% of t = 0 levels at 72 hours when incubated at 37°C. Data representative of n = 3 biological replicates ± SD.

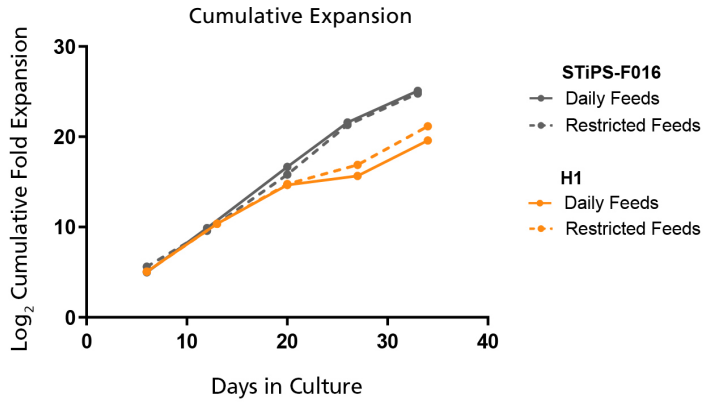
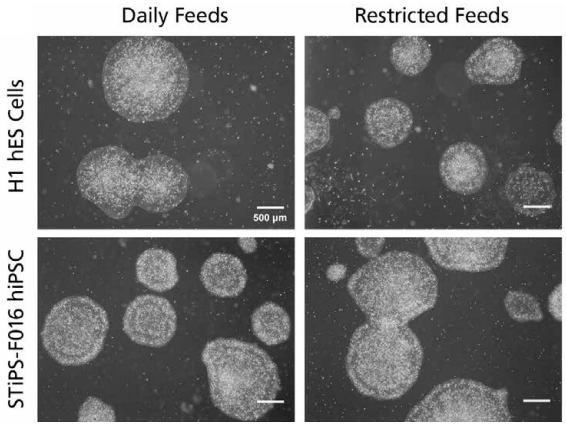


Figure 4. hPSCs Maintained in TeSR™-AOF with Daily and Restricted Feed Schedules Have Comparable Expansion Rates

hPSCs were maintained on Vitronectin XF™ for five passages. At the end of each passage, cell counts were obtained using the Nucleocounter® NC-200 ChemoMetec automated cell counter to count DAPI-stained nuclei. The $\log_2$ transformed cumulative fold expansion was plotted against time in culture (days).



	D0	D1	D2	D3	D4	D5	D6	D7
Restricted	P	-	2F	-	-	F	P / -	P

P = passage, F = feed, 2F = feed with 2X volume

Figure 5. hPSCs Maintained in TeSR™-AOF with Daily and Restricted Feed Schedules Exhibit Comparable Colony Morphology

hPSCs were maintained on Vitronectin XF™ for five passages. Phase-contrast images were taken on day 7 after seeding. For restricted feeds (right panel), hPSCs were fed with a double volume (4 mL) of medium on day 2 after passage, followed by two consecutive skipped days of feeds, with a final single-volume feed (2 mL) on day 5, prior to passaging on day 6 or 7.

FEATURE 4 A Smoother Path to the Clinic

To support cell therapy research, TeSR™-AOF will soon be manufactured under relevant cGMPs with enhanced documentation, including an FDA master file. To learn more about how cGMP-manufactured TeSR™-AOF can support your clinical applications, visit www.stemcell.com/why-tesr-aof. There, you'll find scientific posters and talks, answers to some frequently asked questions, and a form where you can sign up to be notified as soon as TeSR™-AOF manufactured under relevant cGMPs is available.

For more information on how we can support your regulatory needs, including navigating requirements for using TeSR™-AOF in your cell therapy applications, visit www.stemcell.com/regulatory-support or contact your local STEMCELL representative.