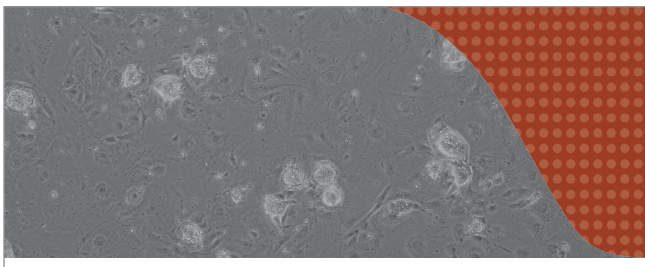

NaïveCult™ Media System

For Transgene-Free Induction and Expansion of Naïve Reset Human Pluripotent Stem Cells

The **NaïveCult™ Media System** consists of defined, induction and expansion media designed for the reversion of primed human pluripotent stem cells (hPSCs) to naïve state and their subsequent expansion.

NaïveCult™ Induction Kit contains defined cell culture media for the chemical induction of transgene-free reset human naïve embryonic stem (ES) and induced pluripotent stem (iPS) cells from the primed state. The kit was developed in collaboration with the Laboratory of Austin Smith, University of Cambridge, UK.[1] This transgene-free culture system works robustly across multiple primed human ES and iPS cells maintained in mTeSR™1 (Catalog #85850) or TeSR™-E8™ (Catalog #05990). Reset naïve hPSCs generated using NaïveCult™ Induction Kit demonstrate uniformly domed, phase-bright colonies with refractive edges and maintain gene expression profiles associated with reset naïve hPSCs.[1-3]

Table 1. NaïveCult™ Induction Kit (Catalog #05580)

PRODUCT	SIZE	CATALOG #
NaiveCult™ Induction Basal Medium	76 mL	05581
NaiveCult™ 20X Induction Supplement A	4 mL	05582
NaiveCult™ 1000X Induction Supplement B	80 µL	05584
NaiveCult™ 5X Induction Supplement C	200 mL	05583
NaiveCult™ 1000X Induction Supplement D	500 µL	05585
NaiveCult™ Expansion Basal Medium	2 x 400 mL	05591
NaiveCult™ 1000X Expansion Supplement	500 µL	05593

NaïveCult™ Induction Kit requires a histone deacetylase inhibitor (HDACi), either sodium butyrate (Catalog #72242) or valproic acid (Catalog #72292), sold separately.

For component storage and stability information, refer to the Product Information Sheet (PIS) for NaïveCult™ Induction Kit (Document #DX21853), available at www.stemcell.com or contact us to request a copy.

Why Use NaïveCult™ Media System?

NAÏVE. Maintains hPSCs with high expression of naïve-associated genes.

TRANSGENE-FREE. No exogenous genes required for reversion to naïve state.

ROBUST. Works consistently across human induced and embryonic stem cell lines.

DEFINED. Formulation contains pre-screened quality components.

NaïveCult™ Expansion Medium allows for the robust expansion of transgene-dependent,[2] transgene-independent[1] and embryo-derived[3] reset hPSCs. Developed under license from the Cambridge Stem Cell Institute at the University of Cambridge, UK, this medium is based on the t2iL + Gö formulation developed by Austin Smith. It is compatible with reset human naïve ES and iPS cells derived using the NaïveCult™ Induction Kit, transgene-induced reset hPSCs,[2] or embryo-derived reset hPSCs.[3] Reset naïve hPSCs cultured in NaïveCult™ Expansion Medium demonstrate uniformly domed, phase-bright colonies with refractive edges and maintain gene expression profiles associated with reset naïve hPSCs.[1-3]

Table 2. NaïveCult™ Expansion Medium (Catalog #05590)

PRODUCT	SIZE	CATALOG #
NaiveCult™ Expansion Basal Medium	400 mL	05591
NaiveCult™ 5X Expansion Supplement	100 mL	05592
NaiveCult™ 1000X Expansion Supplement	500 µL	05593

For component storage and stability information, refer to the PIS for NaïveCult™ Expansion Medium (Document #DX21854), available at www.stemcell.com or contact us to request a copy.

References

1. Guo G et al. (2017) Epigenetic resetting of human pluripotency. Development 144(15):2748-63.
2. Takashima Y et al. (2015) Resetting transcriptional factor control circuitry toward ground-state pluripotency in human. Cell 162(2):452-3.
3. Guo G et al. (2016) Naive pluripotent stem cells derived directly from isolated cells of the human inner cell mass. Stem Cell Reports 6(4):437-46.

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FOR GLOBAL CONTACT DETAILS VISIT OUR WEBSITE

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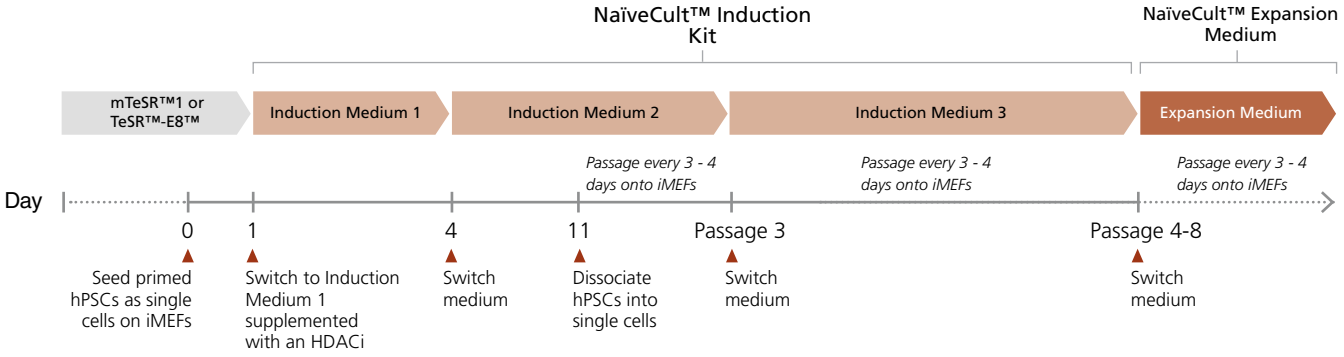

Note: From Day 0 onward, culture under hypoxic conditions (5% O_2, 5% CO_2). Perform full medium changes daily.

Figure 1. Schematic of Reversion of Primed to Naïve hPSCs

Primed hPSCs, grown either in mTeSR™1 or TeSR™-E8™ on Corning® Matrigel®, are plated as single cells on inactivated murine embryonic fibroblasts (iMEFs) and treated with Rho-kinase inhibition (10 µM Y-27632) for 24 hours in hypoxic conditions. On day 1, medium is changed to Induction Medium 1 supplemented with a histone deacetylase inhibitor (HDACi) and cells are cultured for 3 days with daily medium changes. On day 4, medium is changed to Induction Medium 2 and cells are cultured until day 11 with daily medium changes. On day 11, hPSCs are passaged as single cells onto fresh iMEFs and subsequently passaged every 3 - 4 days until the end of passage 2 in Induction Medium 2. From passage 3, hPSCs are cultured in Induction Medium 3. Background differentiation will decrease between passage 3 and 8. At this time cells can be transferred into NaïveCult™ Expansion Medium for long-term maintenance and expansion.

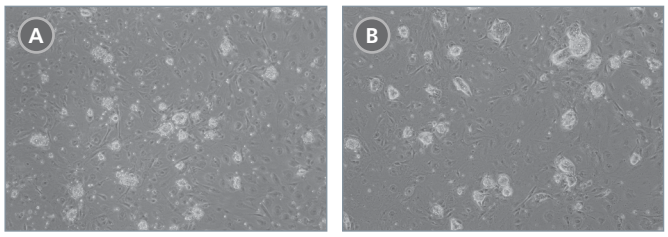

Figure 2. Human ES and iPS Cells Can Be Reverted to a Naïve State

Representative images of human (A) H9 ES cells at passage 7 and (B) WLS-1C iPS cells at passage 9 that were reverted to a naïve state using the NaïveCult™ Induction Kit and subsequently cultured in NaïveCult™ Expansion Medium. During reversion, colonies change from a flat morphology to a tightly packed, domed morphology with refractive edges characteristic of naïve-state hPSCs.

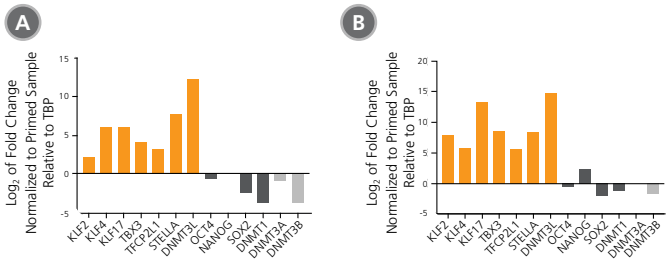

Figure 3. hPSCs Cultured in the NaïveCult™ Media System Express High Levels of Factors Associated with Naïve hPSCs

Gene expression profile of human (A) H9 ES cells and (B) WLS-1C iPS cells reverted using the NaïveCult™ Induction Kit and maintained in NaïveCult™ Expansion Medium.

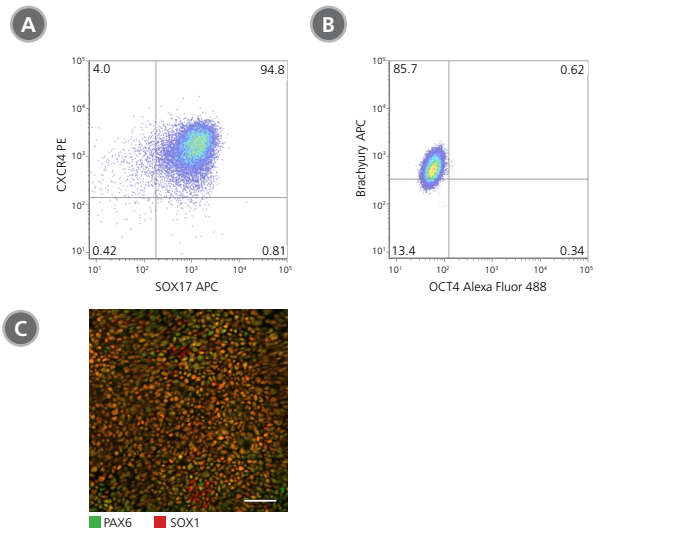

Figure 4. Reset Naïve Human PSCs Cultured in NaïveCult™ Expansion Medium are Capable of Tri-Lineage Differentiation Following Re-Priming

Reset naïve human iPS cells cultured in NaïveCult™ Expansion Medium were re-primed in mTeSR™1 and differentiated to all three somatic lineages. (A) Representative flow cytometry plot of human WLS-1C iPS cells differentiated using the STEMdiff™ Definitive Endoderm Kit (Catalog #05110) demonstrating >90% $CXCR4^+/SOX17^+$ definitive endodermal progenitors. (B) Representative flow cytometry plot of human WLS-1C iPS cells differentiated using STEMdiff™ Mesodermal Induction Medium (Catalog #05220) demonstrating >80% $Brachyury^+/OCT4^-$ mesodermal progenitors. (C) Representative immunofluorescence image of PAX6 (green) and SOX1 (red) double positive neural progenitors derived from human WLS-1C iPS cells differentiated using the STEMdiff™ SMADi Neural Induction Kit (Catalog #08581). Scale bar represents 50 µm.