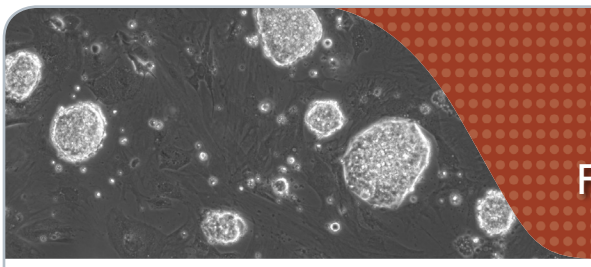
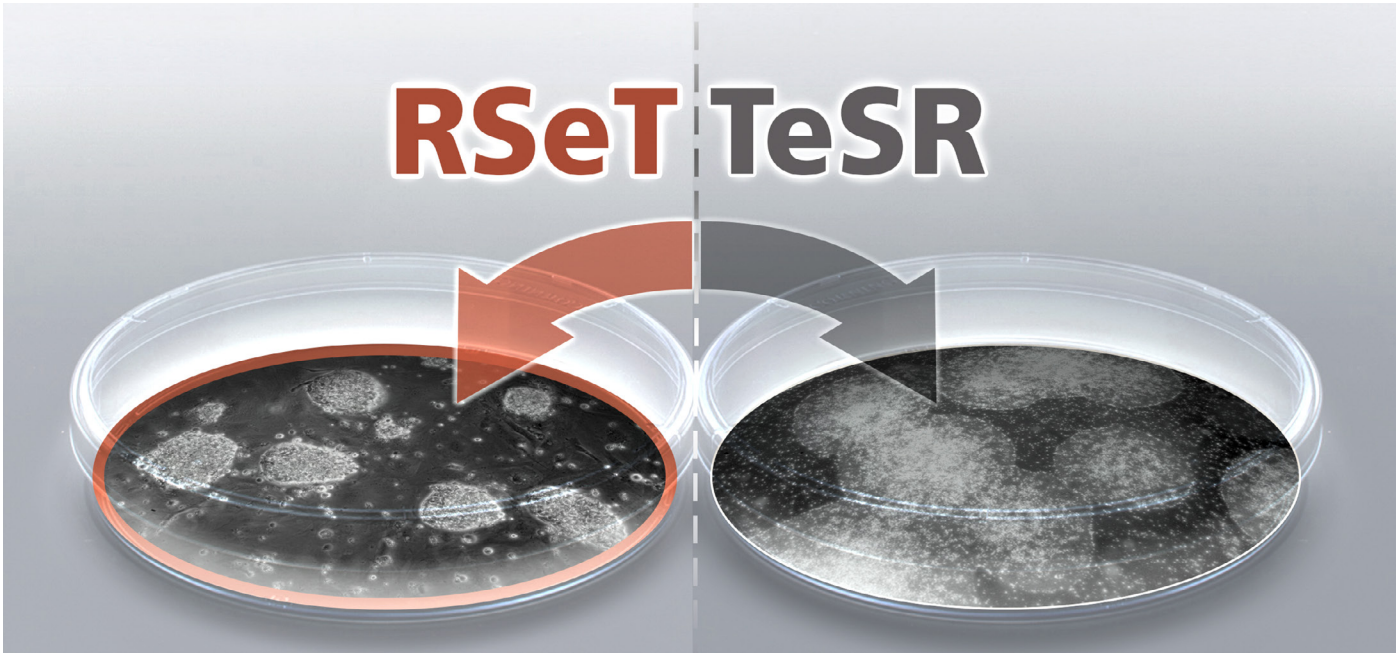


RSeT™

For Naïve-Like Human Pluripotent Stem Cells

RSeT™ is a defined medium that allows researchers to revert human primed pluripotent stem cells to a naïve-like state. RSeT™ also enables researchers to culture human naïve-like pluripotent stem cells long-term on feeders and supports this rapidly expanding field of human pluripotent stem cell (hPSC) research.



PRODUCT	SIZE	CATALOG #
RSeT™ Medium Kit*	500 mL Kit	05978
RSeT™ Basal Medium	400 mL	05969
RSeT™ 5X Supplement	100 mL	05979

* Kit includes the RSeT™ basal medium and supplements.

Advantages:

EASY-TO-USE. Passage as single cells while maintaining normal karyotype.

NAÏVE-LIKE. Maintains pluripotency without inclusion of bFGF or TGFβ.

CONSISTENT. Defined medium contains pre-screened quality components.

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

Spectrum of Human Pluripotent Stem Cell States

During embryogenesis, cells produced within the first few rounds of cell division are typically considered totipotent, as they have the capacity to divide and generate all the tissues of an organism. As embryonic development progresses towards the pre-implantation blastocyst stage, the cells within the inner cell mass are considered pluripotent as these cells are more restricted in their potency and typically gives rise to tissues of the embryo proper. Pluripotency is a dynamic state that exists briefly during embryonic development (Figure 1).

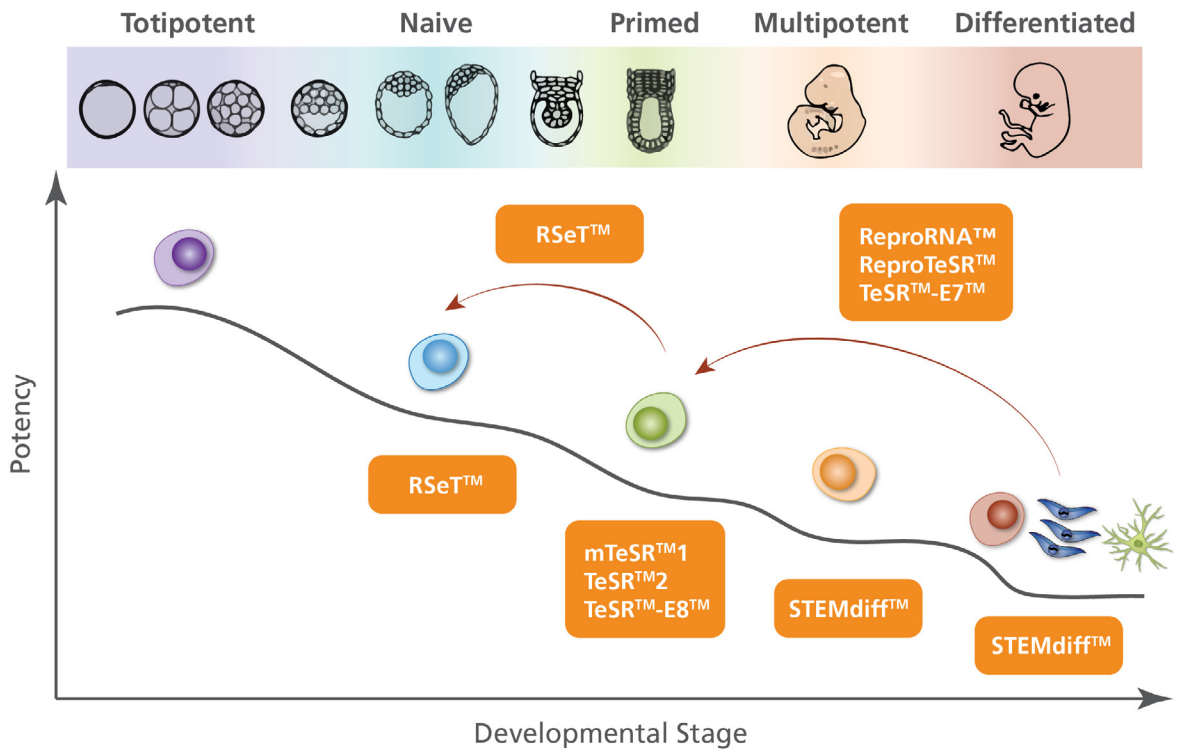


Figure 1. Spectrum of Human Pluripotent Stem Cell States

A schematic illustrating the pluripotency potential of cells at each developmental stage.

Human and mouse pluripotent stem cells are derived from the inner cell mass of the blastocyst embryo. However, gene expression patterns differ between human and mouse pre-implantation epiblasts, and this difference is also reflected in vitro as conventional human pluripotent stem cells (hPSCs) are considered to more closely resemble post-implantation mouse epiblast stem cells (EpiSCs) or primed state than mouse embryonic stem (ES) cells, which are considered to be a more naïve state of pluripotency.[1] Recently, multiple groups have modified culture conditions to revert and maintain hPSCs closer to a naïve-like pluripotency state. However, although global transcriptome analysis shows similarities between naïve-like hPSCs and mouse ES cells, there are still distinct differences in gene expression patterns and different culture requirements.[2-4] While our understanding of human naïve stem cells and their characteristics has improved (Table 1), there are still many unknowns in this rapidly expanding area of hPSC research.

Culture Conditions to Maintain Naïve-Like hPSCs

In vitro, different cell culture media with specific combinations of cytokines or small molecules have been shown to maintain cells in a naïve or primed pluripotent state. Specifically, mouse ES cells are dependent on culture media that contain leukemia inhibitory factor (LIF), while conventional hPSCs and mouse EpiSCs are cultured in FGF and Activin.[2] Multiple research groups have identified different culture conditions capable of shifting and maintaining hPSCs towards the "ground" or "naïve" state and away from the traditional primed state of hPSCs.[3,5-7]

Table 1. Characteristics of Naïve and Primed hPSCs

A comparison of naïve and primed hPSCs and the characteristics that are shared and distinct between these two stem cell states.

	NAÏVE	PRIMED
Colony Morphology	Compact and domed	Compact and flat
Transcriptome	Similar to mouse ES cells	Similar to mouse epiblast stem cells
Cytokines	LIF	bFGF/TGFβ
Clonagenicity	High	Low
Metabolic Activity	Oxidative Phosphorylation/ Glycolysis	Glycolysis
X-Inactivation	XaXa	XaXi or XaXe

RSeT™

RSeT™ is a feeder-dependent, defined medium that reverts primed pluripotent stem cells and maintains cells in a naïve-like state (Figure 2). Developed under license from the Weizmann Institute of Science[5], this improved medium does not contain bFGF or TGFβ. With pre-screened quality components that ensure batch-to-batch consistency, RSeT™ produces robust cultures with phenotypes characteristic of naïve-like stem cells and markers associated with undifferentiated cells (Figures 3-5).

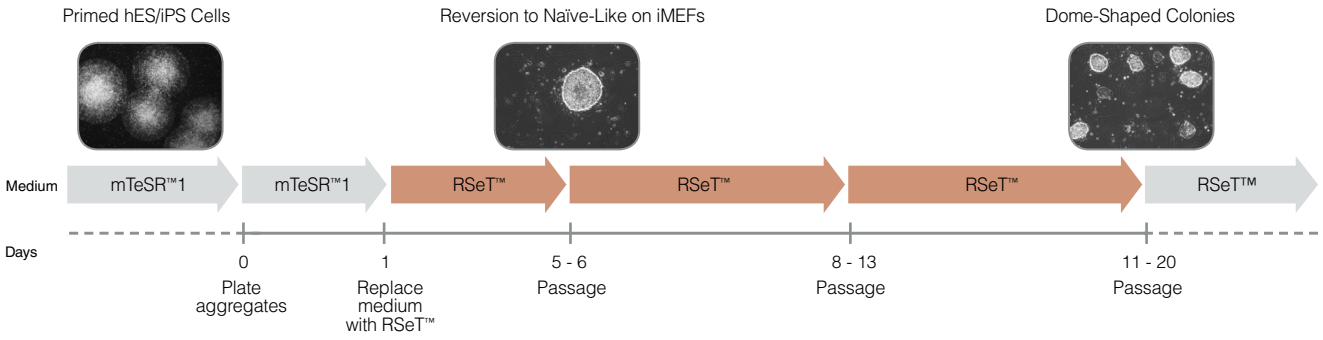


Figure 2. Schematic of Reversion of Primed to Naïve-Like hPSCs

Primed hPSCs are plated as aggregates in mTeSR™1 onto inactivated mouse embryonic fibroblasts (iMEFs). On day 1, mTeSR™1 is replaced with RSeT™, and the medium is exchanged daily. By day 5 or 6, the colonies are generally large enough to be passaged. During the initial culture in RSeT™ medium, colonies expand and begin to adopt domed shape characteristic of naïve-like stem cells and can continue to be propagated in RSeT™.

RSeT™
For Naïve-Like Human Pluripotent Stem Cells

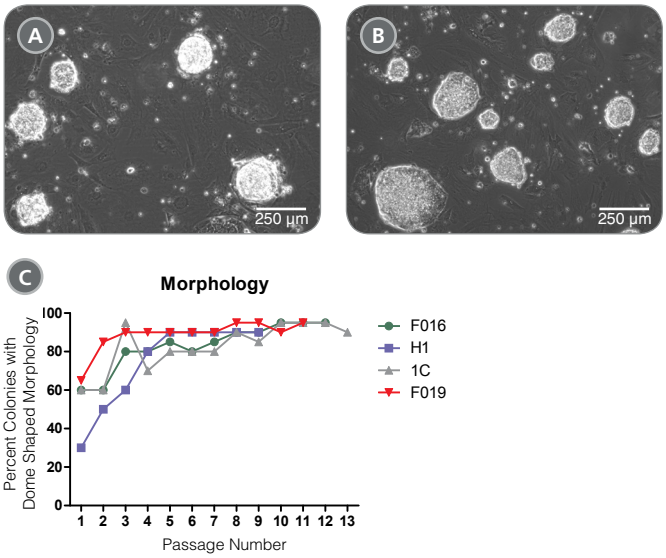


Figure 3. ES and iPS Cells Can Be Reverted to a Naïve-Like State

Representative images of human (A) ES cells (H1) and (B) iPS cells (STiPS-F016) that reverted to a naïve-like state after cultured in RSeT™ for 6 and 10 passages, respectively. (C) During reversion, colonies change from a flat morphology to a domed morphology characteristic of naïve-state hPSCs. Once naïve-like cultures are established, typically >80% of colonies have a dome- shaped morphology.

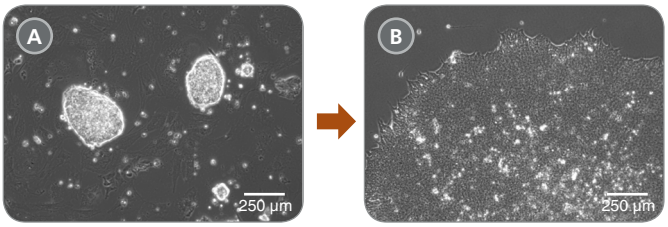


Figure 4. ES and iPS Cells Can Be Converted Back into a Primed State

Representative images of human ES cells (H1) cultured in (A) RSeT™ for 3 passages and then (B) reconverted into a primed state in mTeSR™1 for 5 days.

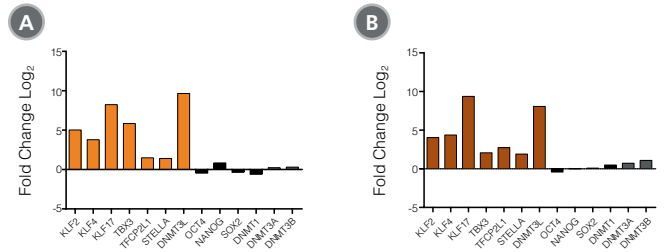


Figure 5. hPSCs Maintained in RSeT™ Express High Levels of Factors Associated with Naïve-Like PSCs

Expression of markers associated with naïve-like PSCs (DNMT3L, KLF17, KLF2, KLF4, NANOG, TBX3, and TCFP2L1) in (A) WLS-1C and (B) STiPS-F019 iPS cells that were reverted to a naïve-like state by culturing in RSeT™. Expression levels were measured by quantitative PCR (qPCR) and normalized to levels in primed STiPS-F019 iPS cells.

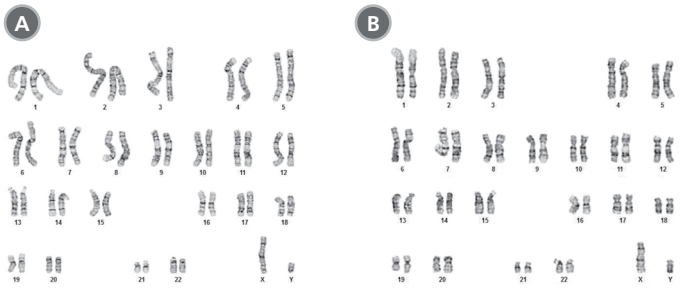


Figure 6. hPSCs Maintained in RSeT™ Display Normal Karyotype after Long-Term Passaging

Representative karyograms of (A) ES cell (H1) and (B) iPS cell (STiPS-F019) lines that were cultured in RSeT™ medium for 31 and 48 passages, respectively.

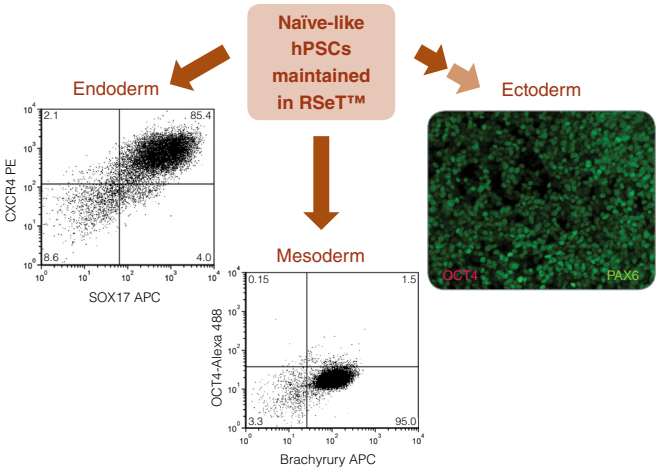


Figure 7. hPSCs Reverted to Naïve-Like State with RSeT™ Medium are Capable of Differentiation to All Three Germ Layers

Human ES cells (H1) maintained in RSeT™ were differentiated into endoderm, mesoderm and ectoderm lineages. Endoderm specification was achieved using STEMdiff™ Definitive Endoderm Kit (Catalog #05110), and mesoderm specification using STEMdiff™Mesoderm Induction Medium (Catalog #05220). Ectoderm specification was achieved by culturing the cells in mTeSR™1 (Catalog #05850) and then in STEMdiff™ Neural Induction Medium (Catalog #05835).

For a complete list of related products, including specialized cell culture and storage media, matrices, antibodies, cytokines and small molecules, visit **www.stemcell.com/hPSCworkflow** or contact us at **techsupport@stemcell.com**.

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