

PRODUCT ORDERING INFORMATION

Stemgent mRNA Reprogramming Method Reagents for 4 wells

Product	Quantity	Product No.	Price
Stemgent[®] mRNA Reprogramming Kit Includes: Stemgent mRNA Reprogramming Factors Set: hOKSM Stemgent Pluriton™ Reprogramming Medium B18R Recombinant Protein	1 kit	00-0071	ASK

Stemgent microRNA Enhanced mRNA Reprogramming Method Reagents for 9-10 wells

Product	Quantity	Product No.	Price
Stemgent[®] mRNA Reprogramming Kit Includes: Stemgent mRNA Reprogramming Factors Set: hOKSM Stemgent Pluriton™ Reprogramming Medium B18R Recombinant Protein	1 kit	00-0071	ASK
Stemgent[®] microRNA Booster Kit Includes: microRNA Reprogramming Cocktail B18R Recombinant Protein	1 kit	00-0073	ASK
Stemgent[®] Stemfect™ RNA Transfection Kit Includes: Stemfect™ RNA Transfection Reagent Stemfect™ Buffer	1 kit	00-0069	ASK
NutriStem [®] XF/FF	01-0005	500mL	ASK
bFGF	RCHEOT002	25 µg	ASK
Nuff Cells	GSC-3002G	4-5×10 ⁶ cells	Available at Cosmo Bio Co., Ltd.
	GSC-3006G		
StainAlive™ TRA-1-60 Antibody (DyLight™ 488), Mouse anti-Human	09-0068	100 µL	ASK
StainAlive™ TRA-1-81 Antibody (DyLight™ 488), Mouse anti-Human	09-0069	100 µL	ASK

Related Products

Product	Quantity	Product No.	Price
Stemgent [®] bFGF, human recombinant	50 µg	03-0002	ASK
Nutristem [®] XF/FF Culture Medium	500 ml	01-0005	ASK
Stemgent [®] StainAlive™ TRA-1-60 Antibody (DyLight™ 488)	100 µl	09-0068	ASK
Stemgent [®] StainAlive™ TRA-1-81 Antibody (DyLight™ 488)	100 µl	09-0069	ASK
Stemgent [®] TRA-1-81 Antibody (Affinity Purified) Mouse anti-Human	100 µl	09-0011	ASK
Stemgent [®] TRA-1-60 Antibody (Affinity Purified) Mouse anti-Human	100 µl	09-0010	ASK
Stemgent [®] Nanog Antibody (Affinity Purified) Rabbit anti-Mouse/Human	100 µl	09-0020	ASK
Stemgent [®] Oct4 Antibody (Affinity Purified) Rabbit anti-Mouse/Human	100 µl	09-0023	ASK
Stemgent [®] SSEA-4 Antibody (Affinity Purified) Mouse anti-Human	100 µl	09-0006	ASK

For a thorough review of the major steps of RNA reprogramming and to download the full protocol, visit www.stemgent.com/rna.



mRNA



microRNA Enhanced

Inquiry

Email : info_jp@reprocell.com

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Fast, Safe and Efficient Non-integrating Reprogramming Technology

CUTTING-EDGE TECHNOLOGY

The Stemgent RNA Reprogramming System includes validated, easy-to-use protocols that reproducibly and efficiently generate iPSC cell lines from both normal and patient-specific cells. The Stemgent mRNA Reprogramming Kit includes synthetically modified mRNAs and soluble B18R to attenuate TLR- and Type I IFN signaling associated with innate, antiviral immunity. Pluriton™ Reprogramming Medium is specifically designed to support mRNA based delivery and reprogramming. The Stemgent kit is formatted to allow tunable cellular expression of individual reprogramming factors transfected into target cells, providing researchers the flexibility to choose the optimal conditions for their experiment.

Stemgent provides two RNA Reprogramming options for iPSC generation; (1) mRNA only reprogramming method (feeder-based format) and the (2) microRNA-enhanced mRNA reprogramming method (feeder-free format). The mRNA only reprogramming method generates primary iPSC cell colonies as quickly as three weeks. The microRNA-enhanced mRNA Reprogramming method improves upon the first generation mRNA Reprogramming Kit, delivering faster reprogramming kinetics, a streamlined protocol and improved efficacy on hard-to-reprogram or refractory cell types. The microRNA-enhanced method combines Stemgent's mRNA Reprogramming Kit with the **microRNA Booster Kit** and the Stemfect™ RNA Transfection Kit to generate expandable primary iPSC cell colonies in as little as 8 days. This new method improves kinetics and reduces mRNA dose per transfection resulting in a per well cost reduction of approximately 35% to 45% versus feeder-based mRNA Reprogramming.

ADVANTAGES

	Stemgent mRNA Reprogramming Method	Stemgent microRNA Enhanced mRNA Reprogramming Method
Reprogramming efficiency	>1%	>1%
Fast Reprogramming Kinetics	Yes	Yes
Somatic Cell → iPSC Cell	3 weeks	2 weeks
Risk of insertional mutagenesis	None	None
Screening for Viral Clearance	None	None
Screening for DNA vector retention and/or integration	None	None
Bio-containment issues associated with viral applications	None	None
# of wells per kit (6 well plate format)	4	9-10 (35%-45% per well cost reduction)
Reprogramming Substrate	Fibroblast feeder layer	Extracellular Matrix
Captures hard-to reprogram and refractory cell types	-	Yes
Simple, user friendly protocol (overnight transfections)	-	Yes

HIGHLY EFFICIENT REPROGRAMMING

The Stemgent RNA Reprogramming System provides efficiencies greater than 1% as compared to other methods, which yield reprogramming efficiencies varying from 0.00001% to 0.01%. In addition to increased yield of colonies and fast reprogramming kinetics, RNA-based reprogramming does not require laborious multi-step downstream passaging or screening for viral vector retention or genomic integration (Table 2).

	Efficiency	Integrating	Screening
RNA	>1%	No	No
Sendai virus	0.01-1%	No	Yes
Episomal/Minicircle	0.0001%	Possible	Yes
Lentivirus	0.001-0.01%	Yes	Yes
Adenovirus	0.0001-0.001%	Possible	Yes
Protein	0.00001%	No	No

Table 2. Stemgent RNA Reprogramming System compared to other reprogramming methods.

Optimized to provide efficiencies greater than 1% over existing reprogramming methods, the Stemgent RNA Reprogramming System generates high-quality iPSC cell lines that do not require additional screening to check for viral vector retention or insertional mutagenesis of the target cell's genome.



FAST GENERATION OF FOOTPRINT-FREE iPS CELL LINES

Reprogramming with RNA produces clinically-relevant iPS cell lines in less than half the time compared to viral and DNA-based reprogramming systems that require additional screening of newly generated iPS cells for the presence of contaminating viral or DNA expression elements. The total time needed to generate a functional, assay-ready iPS cell line using virus-based systems can take up to 25 weeks, whereas the Stemgent RNA Reprogramming System enables the generation of fully characterized and banked ready-to-use iPS cell lines in as little as 8 weeks (Figure 1).

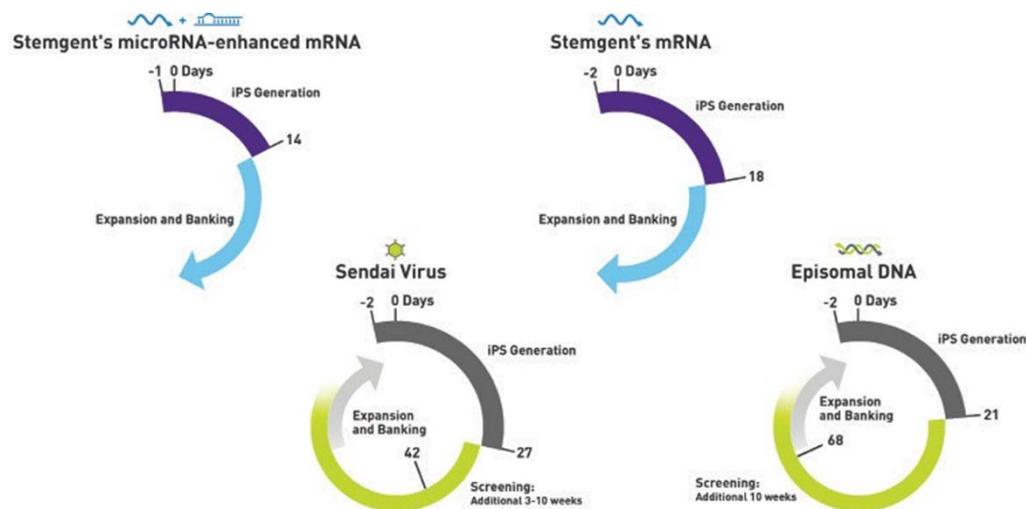


Figure 1. RNA reprogramming experimental timelines compared to other reprogramming systems. The Stemgent RNA reprogramming protocols have been optimized to ensure that human iPS cell colonies are ready to expand in 2-3 weeks – less than half the time than reprogramming with Sendai virus or Episomal DNA vectors which require additional laboratory time for cloning, selecting, and screening of iPS cell lines to confirm that viral and DNA vector expression elements are eliminated during extended passaging. **Note:** Timelines are provided as a guideline for experimental planning and actual timelines can vary based on the cell type and experimental conditions.

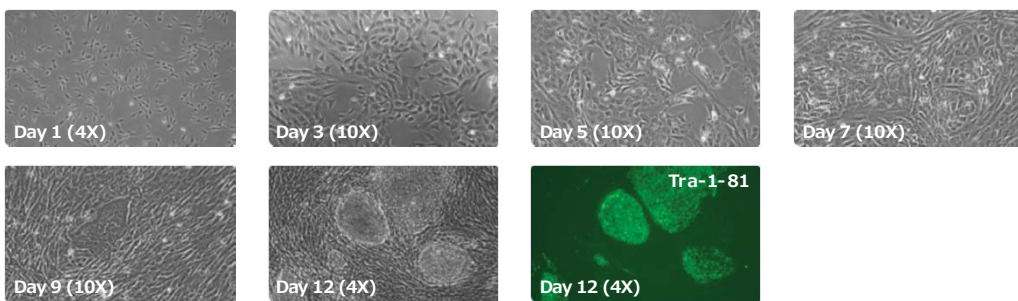


Figure 2. Time-course image progression for the generation of human iPS cells using the Stemgent microRNA Enhanced mRNA Reprogramming System. Phase-contrast images show the progression of morphology changes throughout the reprogramming process. Resulting iPS cell colonies stain positively for TRA-1-81 pluripotency marker expression on Day 12.

HIGH-QUALITY iPS CELL LINES

The RNA Reprogramming System yields footprint-free, stable iPS cell lines. The primary reprogramming cultures produce robust iPS cell colonies that exhibit uniform pluripotency marker expression. The new iPS cell lines are readily established, requiring no cloning or small molecule treatment to eliminate partially reprogrammed colonies (Figure 3).

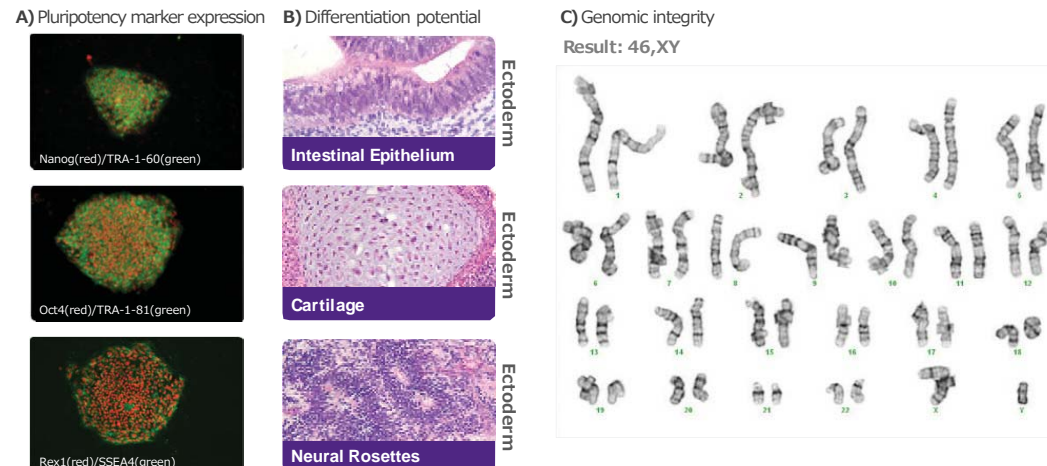


Figure 3. RNA derived iPS cells express pluripotency markers, form teratomas and have a stable karyotype. Human iPS cell lines generated by reprogramming human newborn foreskin fibroblasts with the Stemgent RNA Reprogramming System (A) express the appropriate pluripotency markers, (B) demonstrate distinct teratoma formations with differentiated tissues from all three germ layers (C), and maintain genomic integrity as shown by normal karyotype analysis.

SAFE, NON-VIRAL REPROGRAMMING

Stemgent's RNA Reprogramming System offers advanced, reproducible, generation of human iPS cell lines, with reprogramming efficiencies and kinetics far superior to DNA- and viral-based methods (Figure 4). The RNA Reprogramming System eliminates additional bio-containment and viral vector-associated safety issues, and carries no risk for insertional mutagenesis, an inherent concern with DNA-based reprogramming methods.



Figure 4. Comparison of standard reprogramming methodologies. RNA proves to be the most efficient and safest reprogramming method as compared to all viral- and DNA-based methods.