invitrogen™
by life technologies™

cell reprogramming
transformed

Highly efficient, easy-to-use, non-integrating technology

CytoTune™-iPS Reprogramming Kit

life technologies™

The discovery in 2006 that human and mouse fibroblasts could be reprogrammed to generate induced pluripotent stem cells (iPSCs), with qualities remarkably similar to embryonic stem cells, has created a valuable new source of pluripotent cells for drug discovery, cell therapy, and basic research [1].

Widely accepted methods for cell reprogramming involve using benign viruses to deliver reprogramming factors. The process, however, usually offers reprogramming efficiency rates from 0.00001–0.01% and runs the risk of generating unwanted mutations since the virus must insert itself into the host cell's DNA—essentially altering the genome and leaving a "footprint."

Life Technologies now offers a breakthrough non-integrating, high-efficiency reprogramming technology product for iPSC generation.

What is the CytoTune™-iPS Reprogramming Kit?

The CytoTune™-iPS Reprogramming Kit is used to reprogram somatic cells into induced pluripotent stem cells (iPSCs). This kit utilizes Sendai virus particles containing four genes—Oct3/4, Sox2, Klf4 and cMyc. The expression of these transcription factors in somatic cells has been shown to be a critical factor in the successful generation of iPSCs [1]. Each pack provides enough material to reprogram 10^6 somatic cells at an MOI of 3.

Why should I use the CytoTune™–iPS Reprogramming Kit?

- More efficient—reprogram cells with efficiencies as high as 1%, a 100-fold increase over standard methods [2]
- Integration-free— generate iPSCs with no nuclear remnants of the virus genome
- Easier to use—confidently select colonies after an easy, one-application process

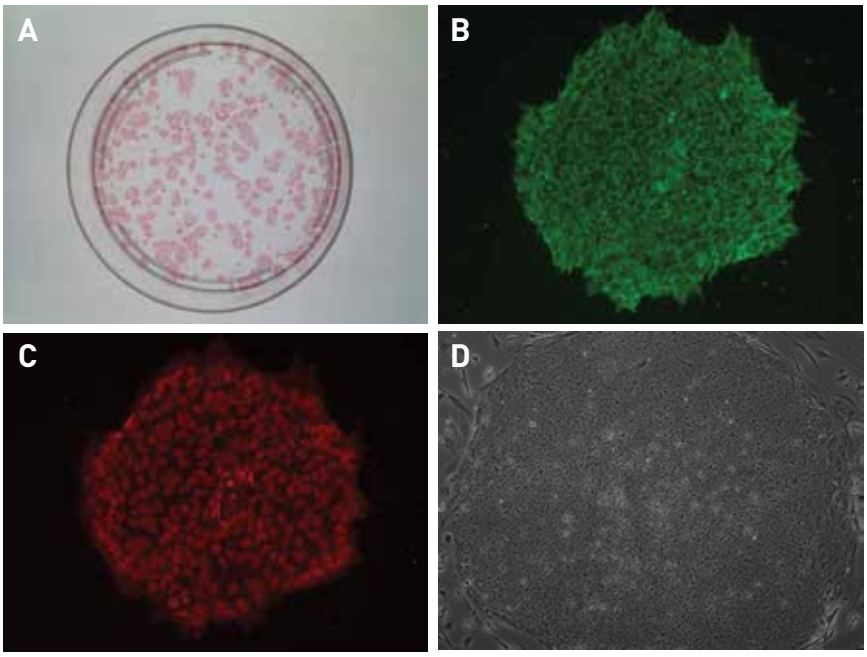
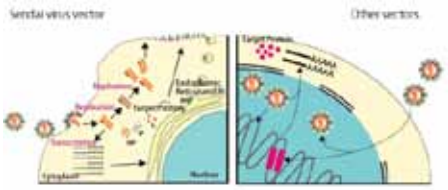


Figure 1. iPSCs generated from BJ fibroblasts using the CytoTune™-iPS Reprogramming Kit. (A) At 4 weeks post transduction, alkaline phosphatase staining of an entire dish. At passage 10, (B) SSEA-4 pluripotent stain 100x, (C) Oct4 pluripotent stain 100x, and (D) phase contrast image of an iPSC colony 100x.

How does the CytoTune™-iPS Reprogramming Kit generate non-integrated iPSCs?

The CytoTune™-iPS Reprogramming Kit includes Sendai virus particles that replicate in the cytoplasm, and do not need to enter the nucleus for transcription. The virus does not integrate into the host genome or alter the genetic information of the host cell.

In addition, iPSCs generated with Sendai virus can be cleared of virus and transgenes via functional temperature sensitivity mutations introduced into the key viral proteins. Other viral reprogramming technologies may contain transgenes that can integrate into the host cell's DNA, potentially disrupting the genome or causing unpredictable results.



How do I use the CytoTune™-iPS Reprogramming Kit?

The Sendai virus particles in the CytoTune™-iPS Reprogramming Kit require only one application to most cell types. Simply add all four particles, containing the four reprogramming genes, to your somatic cells according to the titer of your specific lot, and change the medium every other day for 6 to 7 days before plating them into iPSC medium. Feed and monitor the reprogrammed cells daily until you observe full iPSC colonies. iPSCs may then be passaged routinely. For a full protocol, visit www.invitrogen.com/cytotune.

Which cell types have been reprogrammed with the CytoTune™-iPS Reprogramming Kit?

This kit has been validated for use with BJ (human foreskin fibroblasts) and human blood cells/T cells(3), as well as mouse fibroblasts.

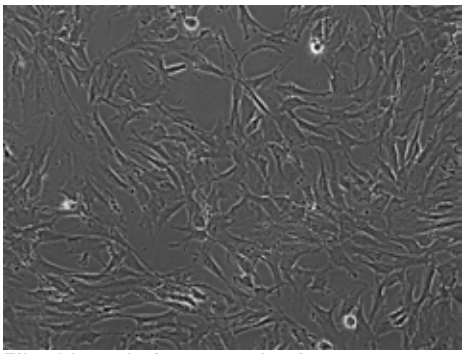
What advantages does the CytoTune™ kit have over other reprogramming technologies such as lentivirus?

The CytoTune™-iPS Reprogramming Kit offers a combination of features that cannot be found in any other reprogramming technology. The Sendai virus offers high efficiency, no integration, as well as single-application reprogramming with most cell lines. The CytoTune™-iPS Reprogramming Kit provides a 100-fold increase in efficiency over standard methods to generate iPS cells, and will not integrate into the host's genome as lentivirus particles do [4].

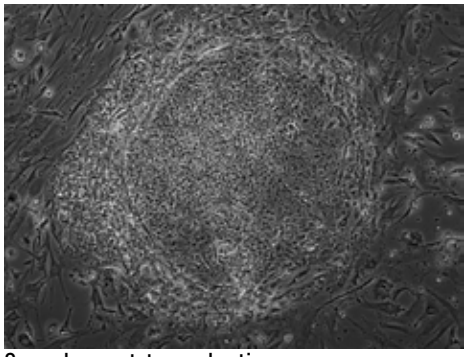
Table 1. Benefits of cell reprogramming using the CytoTune™-iPS Reprogramming Kit

	CytoTune™-iPS Reprogramming Kit (Sendai virus)	Lentivirus/ Retrovirus	Adenovirus	Episomal/Minicircle	Protein	Modified mRNAs
Efficiency	0.01%-1%	0.001-0.01%	0.0001-0.001%	0.0001%	0.00001%	>1%
Integration	No	Yes	No	No	No	No
Multiple transductions/ transfections	No	No	No	No/Yes	Yes	Yes

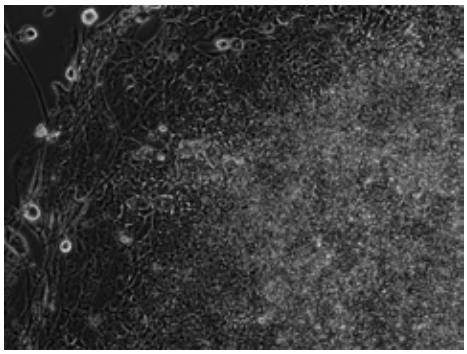
What will my iPSCs look like after I use the CytoTune™-iPS Reprogramming Kit?



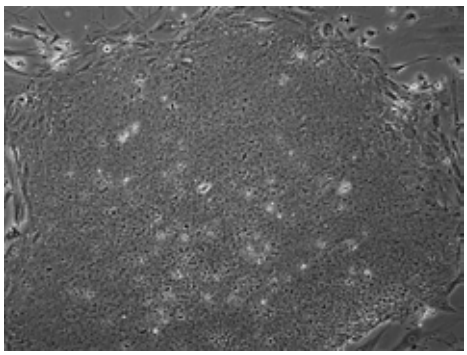
Fibroblasts before transduction



3 weeks post-transduction



4 weeks post-transduction



After manual picking and expansion

Figure 2. Time course images of the generation of iPSCs using the CytoTune™-iPS Reprogramming Kit show the expected morphology of iPSCs—tightly packed colonies with defined borders and a high nucleus-to-cytoplasm ratio.

How do I culture my iPSCs after reprogramming with the CytoTune™ kit?

The culture methods are identical to those for human embryonic stem cells and iPSCs. We recommend using complete KnockOut™ Serum Replacement medium for growth on feeders, but the iPSCs may also be transitioned into feeder-free medium (StemPro® hESC SFM). For the complete details and culturing protocols, visit **www.invitrogen.com/cytotune** for the product manual.

Who do I contact for technical questions?

Our experienced technical support teams are available by phone, fax, or email. Visit www.invitrogen.com/support for your country-specific contact information. Also visit **cellnetwork.community.invitrogen.com** to join our stem cell research network.

How do I know my iPSCs are integration free?

After the reprogramming event, you can confirm that your iPSCs no longer contain remnants of the reprogramming vectors by performing RT-PCR using primers specific for the Sendai virus (see Figure 3).

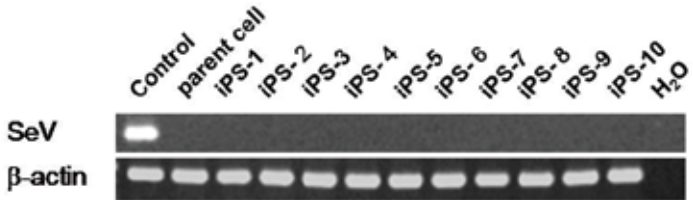


Figure 3.
RT-PCR results of ten iPSC lines generated using the CytoTune™ kit show the absence of the Sendai virus (SeV) after multiple passages.

Ordering Information

Product	Quantity	Cat. No.
CytoTune™ -iPS Reprogramming Kit	1 pack (1 vial of each gene)	A13780-01
CytoTune™ -iPS Reprogramming Kit	3 pack (3 vials of each gene)	A13780-02
iPSC Medium		
KnockOut™ Serum Replacement	500 mL	10828-028
KnockOut™ DMEM/F-12	500 mL	12660-012
NEAA	100 mL	11140-050
GlutaMAX™-1	100 mL	35050-061
Basic FGF	10 µg/mL	PHG-0264
Feeder-free medium		
StemPro® hESC SFM	500 mL	A10007-01
Geltrex™ hESC Qualified Matrix	1 mL	A10480-01
Feeders and feeder medium		
Mouse Embryonic Fibroblasts (Irradiated)	1mL, 1 x 10^6 cells	S1520-100
Attachment Factor	100 mL	S-006-100
ES Cell-Qualified FBS	500 mL	16141-079
DMEM	500 mL	10569-010

References

1. Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126:663–676.
2. Fusaki N, Ban H, Nishiyama A et al. (2009) Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. *Proc Jpn Acad Ser B Phys Biol Sci* 85:348–362.
3. Seki T, Yuasa S, Oda M, et al. (2010) Generation of induced pluripotent stem cells from human terminally differentiated circulating T cells. *Cell Stem Cell* 7:11–14.
4. Zhang X, De Los Angeles A, Zhang J (2010) The art of human induced pluripotent stem cells: the past, the present and the future. *Open Stem Cell J* 2:2–7.

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Headquarters
5791 Van Allen Way | Carlsbad, CA 92008 USA | Phone +1.760.603.7200 | Toll Free in the USA 800.955.6288

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