CytoTune™-iPS 2.0 Sendai Reprogramming Kit and CytoTune™-EmGFP Sendai Fluorescence Reporter FAQs

Product	Qty	Cat. No.
CytoTune™-iPS 2.0 Sendai Reprogramming Kit	1 pack (1 vial of each vector)	A16517
CytoTune™ -iPS 2.0 Sendai Reprogramming Kit	3 pack (3 vials of each vector)	A16518

About CytoTune™-iPS 2.0 Sendai Reprogramming Kit

1. What are induced pluripotent stem cells (iPSCs)?

iPSCs are genetically reprogrammed somatic cells which exhibit a pluripotent stem cell–like state similar to embryonic stem cells. iPSCs can be derived by inducing selected gene expression via various methods including retrovirus-mediated gene transduction and chemical induction.

2. What is the CytoTune™-iPS 2.0 Sendai Reprogramming Kit?

The CytoTune™-iPS 2.0 Sendai Reprogramming Kit is a non-integrating system that uses Sendai virus vectors to reprogram somatic cells into induced pluripotent stem cells (iPSCs). The CytoTune™-iPS 2.0 Sendai Reprogramming Kit contains three CytoTune™ 2.0 reprogramming vectors that are used for delivering and expressing key genetic factors necessary for reprogramming somatic cells into iPSCs. Only one application of the vectors is required for successful reprogramming.

		Volume	
Component	**Cap color**	**A16517**	**A16518**
CytoTune™ 2.0 KOS	clear	100 µL	3 × 100 µL
CytoTune™ 2.0 hc-Myc	white	100 µL	3 × 100 µL
CytoTune™ 2.0 hKlf4	red	100 µL	3 × 100 µL

3. How does the CytoTune™-iPS 2.0 Kit differ from the original kit?

The CytoTune™-iPS 2.0 Kit offers higher reprogramming efficiency, faster clearance of the vectors, and lower cytotoxicity compared to the original kit (Cat. nos. A13780-01, A13780-02). The 2.0 Kit contains three vectors, and one of the vectors in the 2.0 Kit is a polycistronic vector (CytoTune™ 2.0 KOS), which offers an increased reprogramming efficiency. This polycistronic vector has a different backbone containing new temperature sensitivity mutations to the polymerase-related genes, and this helps to clear the virus faster after reprogramming and causes less cytotoxicity to the cells.

4. What does KOS stand for?

KOS is an acronym for the genes h**K**lf4, h**O**ct3/4, h**S**ox2. This is the polycistronic vector, meaning all three of these genes are on one vector.

5. hKlf4 is on the polycistronic vector. Why do I need extra hKlf4?

The polycistrontic configuration of KOS requires that additional polymerase activity be available to compensate for the combination of three genes on one vector. The CytoTune™-iPS 2.0 system uses the extra polymerase from the hKlf4 vector to drive reprogramming in all vectors and enhance reprogramming efficiency. In addition, increased expression of hKlf4 also enhances reprogramming efficiency.

6. Can I optimize the amount of hKlf4 to add?

Yes, the additional Klf4 vector allows the system to be fine-tuned by the user. The MOI of Klf4 can be increased to enhance reprogramming efficiency, or decreased to minimize the total amount of virus.

7. What is Sendai virus (SeV)?

Sendai virus, also known as Hemagglutinating Virus of Japan (HVJ), is a respiratory virus of mouse and rat first isolated in Sendai, Japan in the early 1950s. The virus is classified as mouse parainfluenza virus type I, belonging to the *Paramyxoviridae* family. SeV is an enveloped virus, 150–250 nm in diameter, whose genome is a single chain of (-) sense RNA (15,384 bases). The virus infects cells by attaching to the sialic acid receptor present on the surface of many different cells and is thus able to infect a wide range of cell types of various animal species.

8. Are there any publications that reference the use of the Sendai virus for reprogramming?

To view a list of publications citing the Sendai virus vectors, visit www.thermofisher.com/sendaipubs.

9. How does the Sendai virus (SeV) reprogram somatic cells?

The Sendai virus vectors in the CytoTune™-iPS 2.0 Sendai Reprogramming Kit are based on a modified, non-transmissible form of SeV, which has the Fusion protein (F) deleted. The viral vectors maintain full infectivity to a wide range of cells; however they are no longer capable of producing infectious particles from infected cells because the viral genome lacks the F gene. The Sendai virus vectors contain transgenes that will express factors hOct3/4, hSox2, hKlf4, and hc-Myc. After transduction, the viral vectors will cause the cells to express these four genes, resulting in reprogramming.

10. How is Sendai virus different from lentivirus?

The main difference between Sendai virus and lentivirus is that, when using SeV reprogramming methods, the vectors and transgenes can be eliminated from the cells. Some viruses, like lentiviruses, require integration of viral DNA into the host genome. This can be problematic because this integration is random and can potentially disrupt the function of important genes. Sendai virus requires no integration for viral proteins to be made in the host cell. Other DNA-based viruses, like adenovirus, are non-integrating but must localize inside the nucleus for the viral proteins to be made. This means that there can still be random integration events, where the viral DNA integrates into the host genome. Since Sendai virus is an RNA virus, it does not need to enter the nucleus for transcription. This eliminates the possibility of integration of the transgenes into the host genome.

11. What are the benefits of using an integration-free reprogramming method?

    Integration-free reprogramming methods generate iPSCs that do not contain detectable vectors or transgenes. Traditional technologies used for reprogramming (e.g., lentivirus, retrovirus) integrate into the genome of the target cells. The resulting iPSCs and cells differentiated from those iPSCs will contain foreign DNA and could be unsafe and problematic for use in cell therapy and drug discovery applications. Furthermore, the integration could occur in a critical region of the genome, causing problems with unrelated developmental processes.

12. What cell types have been successfully reprogrammed with Sendai virus?

    The CytoTune™-iPS 2.0 Sendai Reprogramming Kit has been validated for a wide variety of cell types, including human fibroblasts, $CD34^+$ cord blood cells, and Peripheral Blood Mononuclear Cells (PBMCs). For a current list of publications citing the cell types validated using Sendai virus visit www.thermofisher.com/sendaipubs.

13. Can you reprogram mouse cells with this kit?

    Yes, please inquire with technical support for details.

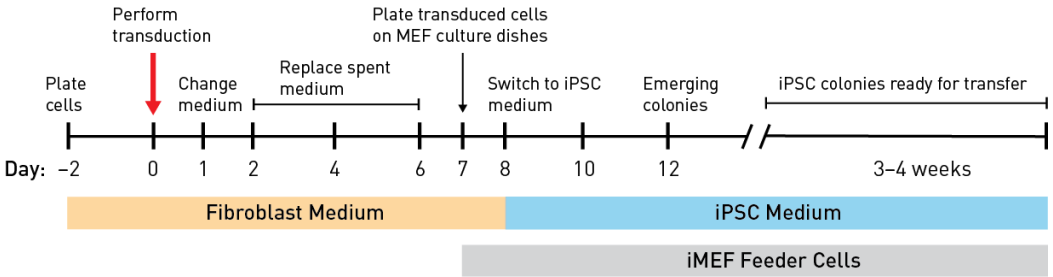
14. Are there safety concerns for Sendai virus?

    Although human is not the natural host for SeV, and the virus is non-pathogenic to humans, appropriate care must be taken to prevent the potential mucosal exposure to the virus. The CytoTune™-iPS 2.0 Sendai Reprogramming Kit must be used under Biosafety Level 2 (BL-2) containment with biological safety cabinet and laminar flow hood, and with appropriate personal safety equipment to prevent mucosal exposure/splash.

How to use the CytoTune™-iPS 2.0 Sendai Reprogramming Kit

15. What does the workflow look like?

    For reprogramming fibroblasts, the workflow is depicted below. For more cell types and also feeder-free options, refer to the user manual:



16. How should I store the CytoTune™-iPS 2.0 Sendai Reprogramming Kit?

    Upon receipt, the kit should be stored at –80°C.

17. Do I need to use all three reprogramming vectors?

Yes, you must use all three reprogramming vectors are used together. Omitting any of the vectors will likely result in little or no reprogramming.

18. Are CytoTune™ 2.0 reprogramming vectors compatible with vectors for the original kit?

CytoTune™ 2.0 reprogramming vectors are **not** compatible with the reprogramming vectors from the original CytoTune™-iPS Reprogramming Kits. Do **not** mix or substitute CytoTune™ 2.0 reprogramming vectors with the reprogramming vectors from the original kits.

19. How many wells of a 6-well plate can I reprogram with one CytoTune™ 2.0 Kit?

One kit (one tube of each vector, i.e., 3 tubes) is sufficient for a minimum of 5 wells of a 6 well dish at MOI of 5:5:3 (KOS, hc-Myc, hKlf4) with a recommended plating density of $2x10^5$ – $3x10^5$ cells/well for human dermal fibroblasts. The virus can only be used once.

20. What does "MOI of 5:5:3 (KOS, hc-Myc, hKlf4)" mean?

MOI (multiplicity of infection) describes the ratio of viral particles to cells. The three vectors in the CytoTune™ 2.0 Kit should each be added to the cells based on an MOI recommendation. We recommend the following MOIs as a starting point, and adjustments can be performed if reprogramming efficiency is not optimal.

Component	MOI
CytoTune™ 2.0 KOS	5
CytoTune™ 2.0 hc-Myc	5
CytoTune™ 2.0 hKlf4	3

21. How do I determine the volume of virus to add to my cells?

An MOI of 5:5:3 (KOS, hc-Myc, hKlf4) is recommended for most cell types. The virus titer varies from lot to lot; the required volume for each MOI is listed on the Certificate of Analysis (CoA) for each lot of product. The CoA can be found at www.thermofisher.com/coa. You may also choose to optimize your MOI as this may vary depending on the cell type. The ratio of KOS and hc-Myc must be 1 to 1, and the MOI of hKlf4 can be varied independently. *For example*: if KOS is 4, then hc-Myc must also be 4.

22. How can I optimize my reprogramming efficiency?

We first recommend increasing the MOI of hKlf4 only. *For example*: go from 5:5:3 to 5:5:6. If optimization is still required, then increase the MOI of KOS and hc-Myc. The ratio of KOS and hc-Myc must be 1 to 1, and the MOI of hKlf4 can be varied independently. *For example:* go from 5:5:3 to 10:10:3 or 10:10:6.

22. How many cells do I need to start my reprogramming experiment with the CytoTune™-iPS Sendai Reprogramming Kit?

    This can vary depending on the cell type. We recommend the following for initial experiments: one to two days before transduction, plate your cells onto wells of a 6-well plate at the appropriate density to ensure that the cells are in the range of 50-80% confluent the day of transduction. Since overconfluency results in decreased transduction efficiency, we recommend replating your cells to achieve a range of 50-80% confluency if your cells have become overconfluent during culturing.

23. Can I scale down my experiments?

    Yes. Initial experiments with fibroblasts have shown than scaling down to a 12-well or 24-well works, but at a potentially reduced efficiency. Cell seeding densities may need to be optimized.

24. What is the optimal passage number for reprogramming patient fibroblasts?

    We recommend reprogramming patient cells at the earliest passage possible. However, it is important to have the cells growing and healthy, which can take between 1–4 weeks. The cells are usually ready to reprogram once they have gone through a total of 3–4 passages.

25. Can I thaw and refreeze the unused virus?

    Avoid repeated freezing and thawing of the reprogramming vectors. Viral titers can decrease significantly with each freeze-thaw cycle and are not guaranteed for kits that have been refrozen or thawed.

26. What is the recommended medium to use for my reprogrammed cells?

    The reprogrammed cells can be grown in KnockOut™ Serum Replacement (KSR) supplemented medium in a feeder-dependent culture, or feeder-free in Essential 8™ Medium. Refer to the user manual for the full protocol:
    https://tools.thermofisher.com/content/sfs/manuals/cytotune_ips_2_0_sendai_reprog_kit_man.pdf

Expected results **with the CytoTune™-iPS 2.0 Sendai Reprogramming Kit**

27. How long does it take to see the iPSC colonies?

    iPSC colonies will begin to form roughly 3 weeks post-transduction. Only one application of the vectors is required for successful reprogramming, enabling selection of iPSC colonies 21–28 days after transduction.

28. What reprogramming efficiency should I expect with this product?

    The CytoTune™-iPS 2.0 Sendai Reprogramming Kit offers reprogramming efficiencies in the range of 0.02- 1.2% with BJ fibroblasts. This may vary for other cell types. Visit www.thermofisher.com/cytotune for more information.

29. Is Valproic Acid (VPA) required when reprogramming using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit?

    We do not recommend using VPA with the CytoTune™-iPS 2.0 Sendai Reprogramming Kit.

30. Should I expect to see cell death during reprogramming?

Depending on your cell type, you should expect to see some cytotoxicity 24–48 hours post-transduction because cytotoxicity is caused by the expression of exogenous genes. However, the new vector backbone included in the CytoTune™ 2.0 Kit has proven to cause less cytotoxicity compared to the original kit, and because only 3 vectors are required, instead of 4 with the original kit, the 2.0 Kit offers reduced cytotoxicity.

31. How will my cells look after I use the CytoTune™ -iPS 2.0 Sendai Reprogramming Kit?

The expected morphology of iPSCs is demonstrated specifically by tightly packed colonies with defined borders and a high nucleus-to-cytoplasm ratio. If you do not observe this morphology or the number of colonies observed is low, then the MOI used for transduction may need to be increased.

32. How can I visualize the iPSCs?

The iPSC colonies can be easily visualized using Alkaline Phosphatase stain, such as the Alkaline Phosphatase Live Stain (Cat. no. A14353). In addition, reprogrammed colonies can be selected utilizing live staining with Tra1-60 or Tra1-81 antibodies that recognize undifferentiated iPSCs and enable the identification of reprogrammed cells from a variety of human cell types. For the complete protocol, visit www.thermofisher.com/cytotune.

33. How do I know when to passage the iPSCs?

iPSCs must be monitored and growth medium must be replaced daily in order to maintain a healthy culture. In general, iPSC colonies should be passaged when the cells reach 70–80% confluence or when most of the colonies are larger than 700 µm. For the complete protocol, visit www.thermofisher.com/cytotune.

34. How many passages does it take to clear the CytoTune™ 2.0 Sendai virus from the generated iPSCs?

It can take as few as five or as many as fifteen passages for the vectors to clear from the cell. This clearance rate is clone-dependent and can be confirmed by PCR or by anti-Sendai antibody. In rare cases, a vector may persist indefinitely. For more information about generating vector-free iPSCs, please refer to the user manual

35. What do I do if my iPSCs still contain the Sendai virus after 10 passages?

The Invitrogen™ CytoTune™-iPS Sendai 2.0 Reprogramming Kit contains a temperature sensitive mutant of c-Myc and KOS that facilitates the clearance of these vectors. To clear c-Myc and KOS incubate the iPSCs at 38–39°C for 5 days. One caveat is that given the sensitive nature of iPSCs, only perform this temperature shift if Sendai virus is in your iPSC lines after more than 10 passages, and you have performed RT-PCR to show that the Klf4 vector is absent from your cells (this vector does not have temperature-sensitive mutations). Then you can perform the temperature shift to remove the c-Myc and KOS vectors.

CytoTune™ -iPS 2.0 Sendai Reprogramming Kit and EmGFP FAQs

Product	Qty	Cat. No.
CytoTune™ EmGFP Sendai Fluorescence Reporter	1 pack	A16519

36. What is the CytoTune™ -EmGFP Sendai Fluorescence Reporter?

The CytoTune™-EmGFP Sendai Fluorescence Reporter is a fluorescent control vector carrying the EmGFP gene. The fluorescent control vector allows the determination of whether a cell line of interest is amenable or refractive to infection by Sendai reprogramming vectors.

37. What is EmGFP?

Emerald Green Fluorescent Protein (EmGFP) is a form of GFP gene with bright expression of green fluorescence used to report the expression of a gene of interest. EmGFP can be seen on standard the FITC channel.

38. How do I use the reporter?

Add the reporter one time to your cells and monitor for expression. We suggest initially transducing your cells with at least 2–3 different MOIs (e.g. 1, 3, and 9). Refer to the user manual for the full protocol at www.lifetechnologies.com/cytotunegfp

39. After addition, when can I see expression of EmGFP?

The expression of EmGFP in successfully transduced cells is detectable at 24 hours post-transduction by fluorescence microscopy, and reaches maximal levels at 48–72 hours post-transduction.

40. Can I mix the reporter with the CytoTune™ -iPS 2.0 Sendai Reprogramming Kit?

If you want to use the EmGFP Reporter with reprogramming, it must be added at the time of reprogramming. Cells infected with Sendai virus will most likely be refractive to further infection. Therefore, do not try to add CytoTune™-iPS 2.0 Sendai Reprogramming Kit to cells already transduced with CytoTune™ -EmGFP Sendai Fluorescence Reporter or vice versa.

Visit our website at www.thermofisher.com/cytotune for more product information, data, protocols, and troubleshooting tips.