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CytoTune®-EmGFP Sendai Fluorescence Reporter

Description

The CytoTune®-EmGFP Sendai Fluorescence Reporter is a fluorescent control vector carrying the Emerald Green Fluorescent Protein (EmGFP) gene. The fluorescent control vector allows the determination of whether a cell line of interest is amenable or refractive to transduction by Sendai reprogramming vectors. 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.

Product	Catalog No.	Amount	Storage*
CytoTune®-EmGFP Sendai Fluorescence Reporter	A16519	100 µL	Store at –80°C.

* Avoid repeated freeze/thaw cycles.

Product Use

For Research Use Only. Not for use in diagnostic procedures.

Important Information

- The titer of the CytoTune®-EmGFP Sendai Fluorescence Reporter is lot-dependent. For the specific titer of the vector, refer to the Certificate of Analysis (CoA) available on our website. Go to **www.lifetechnologies.com/cytotunegfp** and search for the CoA by product lot number, which is printed on the vial.
- Avoid re-freezing and thawing of the CytoTune®-EmGFP Sendai Fluorescence Reporter since viral titers can decrease dramatically with each freeze/thaw cycle.
- Cells that have already been infected with Sendai virus are refractive to further infection by Sendai virus. Therefore, you cannot transduce cells with CytoTune® reprogramming vectors that have already been transduced with the CytoTune®-EmGFP Sendai Fluorescence Reporter or vice versa.

Safety Information

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Culture Conditions

Media: Cell line-dependent

Culture Type: Adherent

Recommended Substrate: Cell line-dependent

Temperature Range: 36°C to 38°C

Incubator Atmosphere: Humidified atmosphere of 5% CO_2. Ensure that proper gas exchange is achieved in culture vessels.

Guidelines for Use

- Transducing your cell line of interest with the CytoTune®-EmGFP Sendai Fluorescence Reporter allows you to determine whether or not the cells can be infected by the Sendai virus vectors. Ability to be transduced by the reporter **does not** indicate the cell line's capability to be reprogrammed.
- You cannot transduce cells that have already been transduced with the CytoTune®-EmGFP Sendai Fluorescence Reporter with CytoTune® reprogramming vectors, and vice versa. If you wish to use the CytoTune®-EmGFP Sendai Fluorescence Reporter during reprogramming, you must add it to the cells at the same time as the reprogramming vectors.
- Different cell types require different MOIs to express detectable levels of EmGFP. As such, cells should be transduced using a range of different MOIs. We suggest initially transducing your cells with at least 2–3 different MOIs (e.g. 1, 3, and 9).
- Expression of EmGFP should be detectable at 24 hours post-transduction by fluorescence microscopy, and reach maximal levels at 48–72 hours.

Transduction Procedure

Day –1 to –2: Prepare the cells for transduction

1. 1–2 days before transduction, plate the cells of interest onto the necessary number of wells of a multi-well plate at the appropriate density to achieve 50–80% confluency on the day of transduction (Day 0). One extra well can be used to count cells for viral volume calculations.
2. Culture the cells for one to two more days, ensuring the cells have fully adhered and extended.

Day 0: Perform transduction

3. On the day of transduction, warm an appropriate volume of cell culture medium for each well to be transduced (e.g., 0.5 mL for each well of a 12-well plate) in a 37°C water bath.
4. Harvest cells from one well of the multi-well plate and perform a cell count. These cells will not be transduced, but will be used to estimate the cell number in the other well(s) plated in Step 1.

   Note: This step is optional and is performed to obtain more accurate MOI calculations. If exact MOIs are not needed, a rough estimate of the number of cells in the well (based on plating density and growth rates) will also suffice.
5. Count (or estimate) the cell number using the desired method (e.g., Countess® Automated Cell Counter), and calculate the volume of the virus needed to reach the target MOI(s). Titer information can be found on the CoA.

$$\text{Volume of virus (µL)} = \frac{\text{MOI (CIU/cell)} \times \text{number of cells}}{\text{titer of virus (CIU/mL)} \times 10^{-3}\ \text{(µL/mL)}}$$

6. Remove one tube of CytoTune®-EmGFP Sendai Fluorescence Reporter from the –80°C storage. Thaw the vector by first immersing the bottom of the tube in a 37°C water bath for 5–10 seconds, and then removing the tube from the water bath and allowing its contents to thaw at room temperature. Once thawed, briefly centrifuge the tube and place it immediately on ice.

Transduction Procedure, continued

7. Add the calculated volume of CytoTune®-EmGFP Sendai Fluorescence Reporter to the pre-warmed cell culture medium prepared in Step 3. Ensure that the solution is thoroughly mixed by pipetting the mixture gently up and down. Complete the next step within 5 minutes.
8. Aspirate the cell culture medium from the cells, and add the solution prepared in Step 7 to the well. Incubate the cells in a 37°C, 5% CO_2 incubator overnight.

Day 1: Replace medium and culture cells

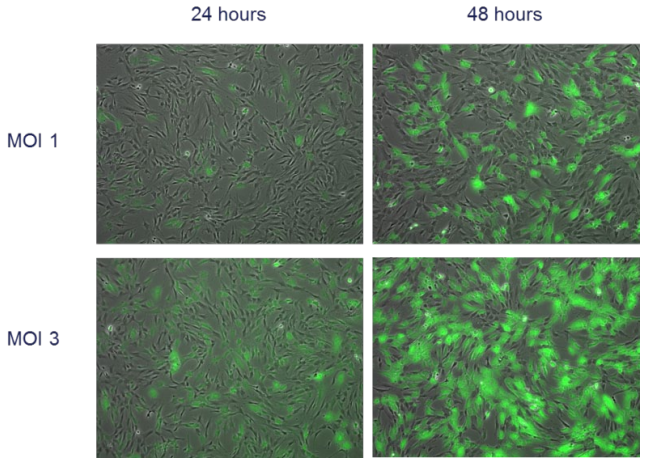
9. 24 hours after transduction, replace the medium with fresh cell culture medium.

   Note: Depending on your cell type, you should expect to see some cytotoxicity 24–48 hours post-transduction, which can affect >50% of your cells. This is an indication of high uptake of the virus. We recommend that you continue culturing your cells and proceed with the protocol.
10. Visualize the cells on a fluorescence microscope using a standard FITC filter set. EmGFP expression should be visible in some cells (expression will reach maximum levels between 48–72 hours).

Day 2+: Replace medium and culture cells

11. 48 hours after transduction, replace the medium with fresh cell culture medium.
12. Visualize the cells on a fluorescence microscope using a standard FITC filter set. EmGFP expression should be much brighter than Day 1, and should be visible in many cells (see Figure 1, below).

Figure 1 BJ HDFn cells transduced with the CytoTune®-EmGFP Sendai Fluorescence Reporter at the indicated MOI (1 or 3) and at the indicated time post-transduction (24 or 48 hours).



Related Products

Product	Cat. No.
CytoTune®-iPS 2.0 Sendai Reprogramming Kit	A16517
CytoTune®-iPS 2.0 Sendai Reprogramming Kit (3 pack)	A16518
CytoTune®-iPS Sendai Reprogramming Kit	A1378001
CytoTune®-iPS Sendai Reprogramming Kit (3 pack)	A1378002

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

			
Temperature limitation	Use by	Batch code	Catalog number
			
Read Safety Data Sheet	Manufacturer		

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Important Licensing Information

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

SeV vectors used in this kit were developed by DNAVEC Corporation (**http://www.dnavec.co.jp**) and their rights for commercial use are the property of DNAVEC Corporation.

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For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit **www.lifetechnologies.com/support**.
For further assistance, email **techsupport@lifetech.com**

www.lifetechnologies.com

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