applied biosystems
by life technologies

TaqMan® iPSC Sendai Detection Kit

Cat. Nos.:	Size:	Store at –15°C to –25°C, protected from light
A13640	75 rxn/assay	
Pub. Part No. A14565PIS	MAN0006755	**Rev. Date:** 23 April 2012

Kit Contents

The *TaqMan*® iPSC Sendai Detection Kit is shipped at ambient temperature and contains the *TaqMan*® Assays listed below. Upon receipt, store the kit at –15°C to –25°C, protected from light.

Assay ID	Target	Amplicon length	Assay context sequence
Mr04269876_mr	Sendai-cMyc	89	GGGTGAATGGGAAGCGGCCGCATGC
Mr04269878_mr	Sendai-OCT3/4	82	TCCCATGCATTCAAACTGACCGTAG
Mr04269879_mr	Sendai-KLF4	74	CACATGAAGAGGCATTTTTAACCGT
Mr04269880_mr	Sendai	59	TGCCCCAAGCAGACACCACCTGGCA
Mr04269881_mr	Sendai-SOX2	62	CACATGTGACCGTAGTAAGAAAAAC

Description

The *TaqMan*® iPSC Sendai Detection Kit is used for detecting the presence and determining the levels of Sendai virus and exogenous transcription factors (OCT3/4, SOX2, KLF4, and cMyc) delivered by the Sendai virus from the CytoTune™-iPS Reprogramming Kit (Cat. nos. A13780-01 and A13780-02). The assays in the *TaqMan*® iPSC Sendai Detection Kit will not detect the corresponding endogenous factors. The detection kit can be used both as a transduction control during reprogramming and as a high-sensitivity detection method for the presence of Sendai virus and transgenes within your iPS clones after reprogramming.

Product Use: For research use only.
Not intended for any animal or human therapeutic or diagnostic use.

Protocol

Brief instructions for using the *TaqMan*® iPSC Sendai Detection Kit are provided below. For detailed instructions, see the *TaqMan*® *Gene Expression Assays Protocol*, available online at **www.lifetechnologies.com/manuals**.

CAUTION! For safety and biohazard guidelines, refer to the "Safety" section in the *TaqMan*® *Gene Expression Assays Protocol*, available online. Before using the *TaqMan*® Universal PCR Master Mix, read the SDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Control Reactions

- We recommend including an un-transduced cell sample from your reprogramming experiment as a negative control to ensure accurate readout of your experiments.
- We recommend including an endogenous control that is shown to work on your cell type to correct for differences in sampling and sample variation. The ideal control is expressed consistently under experimental conditions and is sufficiently abundant across all tissues and cell types studied. For a list of candidate endogenous control assays, refer to the *TaqMan*® *Gene Expression Assays Protocol*, available online at **www.lifetechnologies.com/manuals**.

Step 1: Prepare the cDNA sample to probe the presence of Sendai virus in iPS clones

1. Isolate total RNA from at least 0.5×10^6–1×10^6 cells to ensure sufficient levels of RNA to detect residual virus. We recommend using the TriZol® reagent (Cat. no. 15596) or an Ambion® RNA isolation kit to isolate total RNA.
2. Perform reverse transcription (RT). We recommend using the SuperScript® VILO™ cDNA Synthesis Kit (Cat. no. 11754) or the High Capacity cDNA Reverse Transcription Kit (Cat. no. 4368813 or 4374966). Use the same RT procedure for all samples in your experiment.

   Note: Use at least 1 µg of total RNA for cDNA synthesis to ensure the detection of low-copy-number virus particles.
3. Store the cDNA samples at –15°C to –25°C, if you do not proceed immediately to PCR.

Step 2: Prepare the PCR reaction mix

1. For each sample (to be run in quadruplicate), pipette the following into a nuclease-free 1.5-mL microcentrifuge tube:

PCR reaction mix component	Volume per 20-µL reaction (µL)	
	Single reaction	Four replicates*
TaqMan® iPSC Sendai Detection Assay	1.0	5.0
TaqMan® Universal PCR Master Mix†	10.0	50.0
cDNA template (1–100 ng)‡	4.0	20.0
RNase-free water	5.0	25.0

* Replicate volumes include 20% excess for volume loss from pipetting.

† We recommend using *TaqMan*® Universal PCR Master Mix (Cat. no. 4304437). If you add AmpErase® UNG, the final concentration must be 0.01 U/ µL.

‡ Use the same amount of cDNA for all samples. We recommend that no more than 20% of the PCR be composed of the reverse transcription reaction.

2. Cap the tube, invert it several times to mix, and centrifuge it briefly.

Step 3: Load the plate

1. Transfer 20 µL of PCR reaction mix into each well of a 48-, 96-, or 384-well reaction plate.
2. Seal the plate with the appropriate cover, centrifuge it briefly, and load it into the instrument.

Step 4: Run the plate

1. Create a plate experiment/document for the run using the parameters shown below and run the plate.

Experiment parameters	Stage	Temp. (°C)	Time (mm:ss)
Reaction volume: 20 µL **Ramp rate:** Standard	Hold*	50	2:00
	Hold	95	10:00
	Cycle (40 cycles)	95	0:15
		60	1:00

* Not needed when AmpErase® UNG is not in the reaction.

Step 5: Analyze the results

1. Refer to the user guide for your real-time PCR instrument for instructions on how to analyze your data.

Ordering Information

Individual *TaqMan*® Assays and different sizes of *TaqMan*® Assays are also available. For details on how to order individual *TaqMan*® Assays or different sizes of *TaqMan*® Assays, refer to *TaqMan*® Gene Expression Assays products page at **www.lifetechnologies.com/ordertaqman**.

Documentation and Support

For additional product and technical information, such as Safety Data Sheets (SDS), Certificates of Analysis, etc., visit our website at **www.lifetechnologies.com**. For further assistance, email our Technical Support team at **techsupport@lifetech.com**.

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**.

Purchaser Notification

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

Limited Use Label License: Research Use Only - The purchase of this product conveys to the purchaser the limited, non-transferable right to use the purchased amount of the product only to perform internal research for the sole benefit of the purchaser. No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. This product is for internal research purposes only and is not for use in commercial applications of any kind, including, without limitation, quality control and commercial services such as reporting the results of purchaser's activities for a fee or other form of consideration. For information on obtaining additional rights, please contact **outlicensing@lifetech.com** or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Notice to Purchaser: Limited License - Diagnostic uses of this product require a separate license from Roche. For information on obtaining additional rights, please contact **outlicensing@lifetech.com** or Out Licensing, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008.

Notice to Purchaser: Limited License - Patents covering oligonucleotide conjugates of Minor Groove Binder ("MGB") are owned by ELItech Group and licensed to Life Technologies for use in assays whereby the detection is mediated by a 5' nuclease activity of a polymerase enzyme (the "5'Nuclease Process"). The purchase of this product includes a license to use only this amount of product solely for the purchaser's own use and may not be used for any other commercial use, including without limitation human in vitro diagnostics, repackaging or resale in any form (including resale by purchasers who are licensed to make and sell kits for use in the 5' Nuclease Process). Corresponding products conveying commercial and diagnostic use rights for MGB may be obtained from Life Technologies only under a separate agreement. For information on obtaining additional rights, please contact **outlicensing@lifetech.com** or Out Licensing, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008.