**TaqMan® iPSC Sendai Detection Kit**

**Cat. Nos.:** A13640

**Size:** 75 rxn/assay

**Store at –15°C to –25°C, protected from light**

**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.

<table>
<thead>
<tr>
<th>Assay ID</th>
<th>Target</th>
<th>Amplicon length</th>
<th>Assay context sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mr04269876_mr</td>
<td>Sendai-cMyc</td>
<td>89</td>
<td>GGGTGAATGGGAAGCGGCCGCATGC</td>
</tr>
<tr>
<td>Mr04269878_mr</td>
<td>Sendai-OCT3/4</td>
<td>82</td>
<td>TCCCCATGCATTCAAACTGACCGGTAG</td>
</tr>
<tr>
<td>Mr04269879_mr</td>
<td>Sendai-KLF4</td>
<td>74</td>
<td>CACATGAAGAGGCATTTTTAACCGT</td>
</tr>
<tr>
<td>Mr04269880_mr</td>
<td>Sendai</td>
<td>59</td>
<td>TGCCCCAAGCAGACACCACCTGGCA</td>
</tr>
<tr>
<td>Mr04269881_mr</td>
<td>Sendai-SOX2</td>
<td>62</td>
<td>CACATGTGACCGGTAGTAAGAAAAAC</td>
</tr>
</tbody>
</table>

### 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 iPSC 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:

<table>
<thead>
<tr>
<th>PCR reaction mix component</th>
<th>Volume per 20-µL reaction (µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Single reaction</td>
</tr>
<tr>
<td>TaqMan® iPSC Sendai Detection Assay</td>
<td>1.0</td>
</tr>
<tr>
<td>TaqMan® Universal PCR Master Mix†</td>
<td>10.0</td>
</tr>
<tr>
<td>cDNA template (1–100 ng)‡</td>
<td>4.0</td>
</tr>
<tr>
<td>RNase-free water</td>
<td>5.0</td>
</tr>
</tbody>
</table>

* 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.

<table>
<thead>
<tr>
<th>Experiment parameters</th>
<th>Stage</th>
<th>Temp. (°C)</th>
<th>Time (mm:ss)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reaction volume:</strong></td>
<td>Hold*</td>
<td>50</td>
<td>2:00</td>
</tr>
<tr>
<td><strong>20 µL</strong></td>
<td>Hold</td>
<td>95</td>
<td>10:00</td>
</tr>
<tr>
<td><strong>Ramp rate:</strong></td>
<td>Cycle</td>
<td>95</td>
<td>0:15</td>
</tr>
<tr>
<td><strong>Standard</strong></td>
<td>(40 cycles)</td>
<td>60</td>
<td>1:00</td>
</tr>
</tbody>
</table>

* 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.

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