Protocol-at-a-Glance

Pluripotency Check PCR Primer Set
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(PT5086-2)

The **Pluripotency Check PCR Primer Set** (Cat. No. 631966) consists of 16 forward and reverse primer mixes, based on nine genes expressed in pluripotent stem cells and two control genes. It can be used to validate the pluripotent status (ability to differentiate into specialized cells of all three germ layers) of your stem cell culture.

Attention

Note: This protocol has been optimized for use with the NucleoSpin® RNA II Kit, SMART™ MMLV Reverse Transcriptase, and the TITANIUM® *Taq* PCR Kit. These reagents are sold together with the Pluripotency Check PCR Primer Set as the **Pluripotency Check PCR Kit** (Cat. No. 631965). If you plan to use different reagents for RNA extraction, reverse transcription, and/or PCR, you will need to optimize the amount of template and the PCR conditions described below.

Protocol:

1. Perform total RNA extraction.
   We recommend using the NucleoSpin RNA II Kit (Cat. No. 740955.10).
2. Perform first-strand cDNA synthesis. Use 2 µg total RNA and 2.5 µM oligo(dT) primer in a 20 µl reaction. Dilute the resulting first-strand cDNA 50X with water.
   We recommend using SMART MMLV Reverse Transcriptase (Cat. No. 639523).
3. Set up the PCR reactions by adding each of the components listed in Table I.

Table I. PCR Reagent Volumes using TITANIUM PCR Kit (Cat. No. 639211)*

PCR-Grade H_2O	36 µl
10X PCR Buffer	5 µl
50X dNTP (10mM each)	1 µl
50X TITANIUM Enzyme	1 µl
Primer Mix (10 µM each)	2 µl
Diluted 1st-strand cDNA	5 µl (corresponding to 10 ng RNA)
Total volume	50 µl

Attention

*If you use different reagents for RNA extraction, reverse transcription and PCR, you will need to optimize the amount of template and the PCR conditions.

4. Begin thermal cycling using the following cycling parameters:
   - 94°C for 3 min
   - 30 cycles
     94°C for 30 sec
     68°C for 1 min
   - 68°C for 3 min
   - Hold at 4°C
5. Analyze 5 µl of each reaction by electrophoresis on a 1.5% agarose/TAE/EtBr gel, along with an appropriate DNA size marker. Please see page 2 for expected amplicon sizes.

Clontech

United States/Canada
800.662.2566
Asia Pacific
+1.650.919.7300
Europe
+33.(0)1.3904.6880
Japan
+81.(0)77.543.6116

Clontech Laboratories, Inc.
A Takara Bio Company
1290 Terra Bella Ave.
Mountain View, CA 94043
Technical Support (US)
E-mail: tech@clontech.com
www.clontech.com

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Amplicons of the following sizes should be observed:

Oct3/4 (Total)	~485 bp	Klf4 (Total)	~739 bp
Oct3/4 (Endogenous)	~224 bp	Klf4 (Endogenous)	~206 bp
Nanog (Total)	~364 bp	Ecat1	~164 bp
Nanog (Endogenous)	~223 bp	ERas	~210 bp
Sox2 (Total)	~193 bp	Esg1	~376 bp
Sox2 (Endogenous)	~297 bp	Rex1	~287 bp
c-Myc (Total)	~228 bp	ß-Actin	~603 bp
c-Myc (Endogenous)	~170 bp	GAPDH	~568 bp