Product Analysis Certificate

PRODUCT	**Cas9 RT-PCR primer set**
CATALOG #	**CAS9-PR-1**
LOT #	**141201**
STORAGE	**-20°C (long term)**
	SHELF LIFE 12 months from date of receipt with proper storage
SHIPPING	**Room temp/Blue ice/Dry ice**

DESCRIPTION

The type II prokaryotic CRISPR (clustered regularly interspaced short palindromic repeats) adaptive immune system has been shown to facilitate RNA-guided sequence-specific DNA cleavage, which provides a new class of genome engineering tools. To make the RNA-directed Cas9 system more efficient, affordable, and convenient to use, SBI has developed a suite of PrecisionX™ Cas9 products http://www.systembio.com/cas9 . In order to help customer validate the expression of Cas9 in cells, we designed two sets of RT-PCR primer for this purpose. The amplicon for primer set 1 is 219bp and amplicon for primer set2 is 122bp. These primer sets allow for detection of Cas9 nuclease, Cas9 Nickase and Cas9 double mutant at messenger level. They are good for validation of Cas9 expression constructs from SBI and those from Dr. Feng Zhang's lab as well.

PACKAGE CONTENTS for CAS9-PR-1 Primer set

Cat#	Description	Amount	Amplicon size
CAS9-PR-1	**Cas9 RT-PCR primer set1**	**50 µl (5 µM)**	**219bp**
CAS9-PR-1	**Cas9 RT-PCR primer set2**	**50 µl (5 µM)**	**122bp**

Protocol and Validation Data

1) Day 1, seed 293 cells at 1x10^5 in 12 well plate
2) Day2 , transfect 293 cells with 1ug CAS9 expression vector (in this study CASLV105PA-1)
3) Day 5, extract RNA from transfectant cells and non transfectant negative control cells
4) Treat the RNA sample with DNase I, and convert RNA into cDNA. We recommend that first time users start with 1 ug of total RNA. Please follow the protocol of the cDNA synthesis provided by the manufacturer. Dilute the resulting cDNA in 100ul with nuclease-free H_2O.
5) Set up qPCR reaction as follow. To exclude the possibility of plasmid DNA contamination in your RNA sample, you also run addition reactions using RNA sample.

qPCR reaction	
2X SYBR Green qPCR Mastermix buffer	10ul
Primer set (5uM)	1ul
cDNA	0.5ul
Nuclease-free water	Up to 20ul

6) Real-time qPCR Instrument Parameter
Follow the guideline as detailed for your specific Real-time instrument. The following parameters tested by SBI were performed on an Applied Biosystems 7900 Real-time PCR system, but can also apply to an ABI7500 or 7300 system.

Instrument setup
qPCR cycling program
1. 50°C 2min
2. 95°C 10min
3. 95°C 15sec
4. 60°C 1min

40 cycles of step 3 and 4

An additional recommendation is to include a melt analysis after the qPCR run to assess the Tm of the PCR amplicon to verify the specificity of the amplification reaction. Refer to the User Manual for your specific instrument to conduct the melt analysis and the data analyses of the amplification plots and Cycle Threshold (Ct) calculations. In general, Cycle Thresholds should be set within the exponential phase of the amplification plots with software automatic baseline settings.

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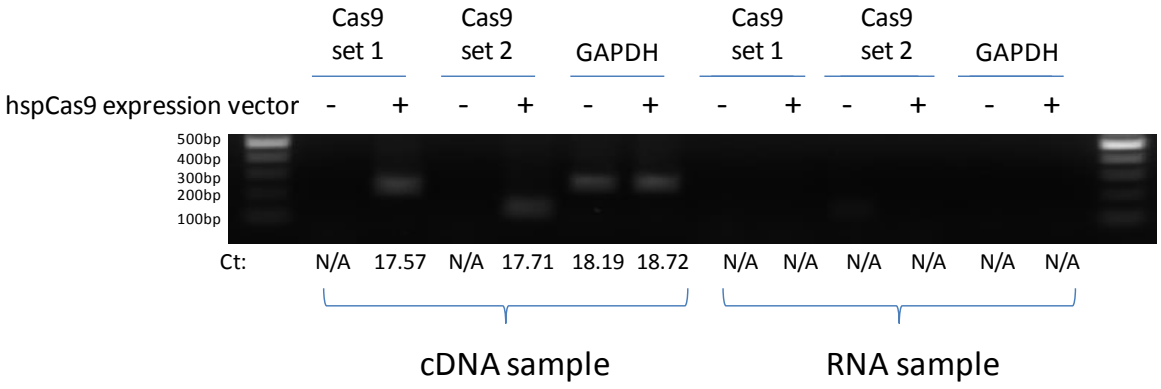
7) Data show below are end products separated on a 1.5% agarose gel stained with ethidium bromide for cas9 transfected cells and non-transfected cells. The CT value for Cas9 transfected cells is around 17 and is undetectable in non-transfected negative control cells as well as RNA samples.



Important Licensing Information

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