

Upload image  
2019

Working

## Quantitative RT-PCR using the TaqMan® Gene Expression Assays on StepOnePlus™ Real-Time PCR System

Lisa-Maria Rosenthal<sup>1</sup>, Giang Tong<sup>1</sup>, Katharina Schmitt<sup>1</sup>

<sup>1</sup>Deutsches Herzzentrum Berlin

[dx.doi.org/10.17504/protocols.io.2v8ge9w](https://doi.org/10.17504/protocols.io.2v8ge9w)



Lisa-Maria Rosenthal  
Deutsches Herzzentrum Berlin



### ABSTRACT

## Quantitative RT-PCR using the TaqMan® Gene Expression Assays on StepOnePlus™ Real-Time PCR System

### MATERIALS

NAME	CATALOG #	VENDOR
TaqMan® Gene Expression Assay	4331182	Thermo Fisher Scientific
StepOnePlus™ Real-Time PCR System	4376600	Thermo Fisher Scientific

1

- Allow the TaqMan® Gene Expression Assay components to thaw on ice

2 Primers

Primers			
Gene	Gene Assay ID	Gene	Gene Assay ID

RBM3	Hs00943160_g1	CIRP	Hs00989762_g1
IL-6	Hs00174131_m1	MCP-1 (Ccl2)	Hs00234140_m1
iNOS	Hs01075529_m1	GAPDH	Hs02786624_g1

P

- 3 ■ Prepare the TaqMan® master mix on ice.

Component	Volume (μL)
Gene Assay Primers	0.5
TaqMan Gene Expression PCR Master Mix	5.0
cDNA	0.5
Nuclease-free H <sub>2</sub> O	4.0
Total per reaction	10.0

- 4 ■ Run the StepOnePlus™ Real-Time PCR System under the following conditions.

Step	Times and Temperatures			
2. PCR Step	Initial Steps		PCR (40 Cycles)	
	AmpErase ® UNG Activation	AmpliTaq Gold® DNA Polymerase Activation	Melt	Anneal/ Extend
	HOLD	HOLD	CYCLE	
	2 min @ 50 °C	10 min @ 95 °C	15 sec @ 95 °C	1 min @ 60 °C



This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited