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

PLOS One

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ABSTRACT

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

MATERIALS

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TaqMan® Gene Expression Assay **Thermo Fisher Scientific Catalog #4331182**

StepOnePlus™ Real-Time PCR System **Thermo Fisher Scientific Catalog #4376600**

Protocol status: Working
We use this protocol and it's working

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- 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
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- 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 ₂ 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	Initial Steps		PCR (40 Cycles)	
	AmpErase® UNG Activation	AmpliTaq Gold® DNA Polymerase Activation	Melt	Anneal/Extend

Step	HOLD	HOLD	CYCLE	
	2 min @ 50 °C	10 min @ 95 °C	15 sec @ 95 °C	1 min @ 60 °C