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

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

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Abstract

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

Materials

MATERIALS

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

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

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

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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 H2O	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