One-Step GoldNStart TaqGreen RT-qPCR Master Mix, 4x (GTGRTMM)

Catalog #MB6028-1, 1 mL
or
Catalog #MB6028-5, 5 mL

Introduction

ScienCell's One-Step GoldNStart TaqGreen RT-qPCR master mix, 4x (GTGRTMM) is designed for sensitive RNA reverse transcription and quantification by SYBR® Green dye-based reverse transcription quantitative PCR (RT-qPCR). The ready-to-use 4x master mix contains UNG/dUTP (to eliminate PCR carryover contamination), engineered M-MLV reverse transcriptase with "tepid-start" property, Taq DNA polymerase with "hot-start" property, dNTPs, a buffer system for robust reverse transcription and qPCR, and an inert gold-color loading indicator (ScienCell, catalog #GQ300G). The "tepid-start" and "hot-start" property achieved through ScienCell's unique chemically modified M-MLV reverse transcriptase and Taq DNA polymerase provides maximal inhibition of primer dimer formation. The advanced buffer formulation provides superior specificity and efficiency with a wide linear dynamic range. The inert gold-color loading indicator allows for better visualization and tracking of sample loading in qPCR plates or tubes.

Kit Components

Catalog #MB6028-1

Cat #	Item	Quantity	Storage
MB6028a-1	One-Step GoldNStart TaqGreen RT-qPCR Master Mix, 4x	1 mL	-20°C
MB6028b-1	Nuclease-free water	1 mL	4°C

or

Catalog #MB6028-5

Cat #	Item	Quantity	Storage
MB6028a-1	One-Step GoldNStart TaqGreen RT-qPCR Master Mix, 4x	1 mL x 5	-20°C
MB6028b-1	Nuclease-free water	1 mL x 5	4°C

Quality Control

The linear dynamic performance of GTGRTMM is verified with serially diluted total RNA samples. RNase and DNase activity were NOT detected by incubating each component of GTGRTMM with single-stranded and double-stranded DNA at 37 ºC for 4 hours.

Product Use

GTGRTMM is for research use only. It is not approved for human or animal use, or for application in clinical or *in vitro* diagnostic procedures.

Shipping and Storage

The product is shipped on dry ice. Upon receipt, store One-Step GoldNStart TaqGreen RT-qPCR Master Mix, 4x (Cat #MB6028a) at -20ºC in a manual defrost freezer and nuclease-free H_2O (Cat #MB6028b) at 4ºC. Aliquot as needed. Avoid repeated freeze-and-thaw cycles.

Procedure

Important: *Only use nuclease-free reagents in PCR amplification.*
Note: *This master mix does not contain a ROX passive reference dye. If the qPCR instrument being used has a "ROX passive reference dye" option, please deselect this option.*
Note: *Do NOT warm the master mix to above 40°C before using, otherwise the reverse transcriptase will be activated and may lead to primer-dimer formation.*

1. Thaw One-Step GoldNStart TaqGreen RT-qPCR Master Mix and place on ice.

2. Prepare 20 µL qPCR reactions in qPCR tubes or plates as shown in Table 1. For other reaction volume setups, scale up or down proportionally.

Table 1. Preparation of 20 µL RT-qPCR reactions

Component	Volume	Final concentration
One-Step GoldNStart TaqGreen RT-qPCR master mix	5 µL	1x
mRNA or total RNA	variable	-
Nuclease-free water	variable	-
Forward and reverse primers	variable	250 – 500 nM
Total volume per reaction	20 µL	-

3. Seal the qPCR reaction wells. Centrifuge the tubes or plates at 1,500X g for 15 seconds. For maximum reliability, a minimum of 3 replicates are recommended

4. Refer to Table 2 for a typical RT-qPCR program setup. Adjust properly according to the optimized RT-qPCR conditions for the reactions to run. Load the PCR tubes or plates into the qPCR instrument and run the program.

Table 2. A typical RT-qPCR program setup

Step	Temperature	Time	Cycles
Reverse transcription	50°C	20 min	1
Taq DNA polymerase activation	95°C	10 min	1
Denaturation	95°C	20 sec	30-40
Annealing	50 - 68°C	20 sec	
Extension	72°C	20-45 sec	
Data acquisition	Plate read		
Optional	Melting curve analysis		1
Hold	20°C	Indefinite	1

5. For data analysis, please refer to the data analysis software of the qPCR instrument being used.