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Contact & Technical support

Tel.: 0731 3608 123
Fax: 0731 3608 962
e-mail: info@genaxxon.com

GENAXXON bioscience

Genaxxon bioscience GmbH
Sölflinger Str. 70
D-89077 Ulm

www.genaxxon.com

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HotScriptase RT

Hotstart reverse Transcriptase and DNA Polymerase

Mastermix for parallel reverse transcription and PCR (realtime PCR) without isothermal transcription step.

Cat#: M3057
Cat#: M3062
Cat#: M3063
Cat#: M3064

Version: 080317

Protocol using HotScriptase RT Mastermixes – cat#: M3057/M3062/M3063/M3064

This protocol serves as a guideline for PCR and RT-PCR. Optimal reaction conditions such as incubation times, temperatures and amount of template DNA or RNA may vary and must be determined individually.

We recommend to use RNA-specific primers, as HotScriptase RT will amplify from any nucleic acid target consisting of both RNA or DNA. We recommend using an RNA extract as a positive control, and the Taq DNA polymerase Mix (included) as a negative control to confirm no amplification of DNA.

RNA-specific primers are binding on exon-exon junctions. For instance, you can simply use one of the free primer design tools in the internet, such as primer-blast on the homepage: www.ncbi.nlm.nih.gov/tools/primer-blast/. Ensure that you select the option: "primers must span an exon-exon junction". Primers designed with this parameter will limit amplification to mRNA.

HotScriptase RT Mastermix can also be used for realtime PCR:
M3057: HotScriptase RT Mastermix with GreenDye and ROX is suitable for all realtime dye-based qPCR assays requiring a passive reference dye.
M3062: HotScriptase RT Mastermix with GreenDye, without ROX is suitable for all realtime dye-based qPCR assays.
M3063: HotScriptase RT Mastermix Probe with ROX is suitable for all probe-based qPCR assays requiring a passive reference dye.
M3064: HotScriptase RT Mastermix Probe without ROX is suitable for all probe-based qPCR assays.

HotScriptase is especially well suited for a combined RT-PCR with fluorescent dye or probes as no isothermal step has to be implemented and no additional pipetting step.

Important notes before getting started

- Set up all reaction mixtures in an area separate from that used for DNA preparation or PCR product analysis.
- Use disposable tips containing hydrophobic filters to minimize cross-contamination.
- Genaxxon HotScriptase RT is **optimized for an amplicon size between 60- 400 bp.**

Procedure

1. **Thaw primer solutions**
   Keep on ice after complete thawing, and mix well before use.

   Optional: Prepare a primer mix of an appropriate concentration using sterile, bidest water. This is recommended if several amplification reactions using the same primer pair are to be performed. The final volume of diluted primer mix plus the template DNA, added at step 4, should not exceed 12.5µL per reaction.

2. **Thaw HotScriptase RT Mastermix (2X)** at RT or on ice.
   Keep the solutions on ice after complete thawing. It is very important to mix the HotScriptase RT Mastermix well before use to avoid local differences in salt concentration. The Genaxxon bioscience HotScriptase RT Mastermix is provided as a 2X concentrated (i.e. a 12.5µL volume of the mastermix is required for RT-PCR reactions with a final volume of 25µL). For volumes smaller than 25µL, the 1:1 ratio of mastermix to diluted primer mix, template DNA and water should be maintained. A negative control (RT-PCR without RNA or template DNA) should be included in every experiment. It is recommended that the PCR tubes are kept on ice until they are placed in the thermal cycler.

3. **Distribute the appropriate volume of diluted primer mix into the PCR tubes containing the HotScriptase RT Mastermix.**

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Procedure using HotScriptase RT Mastermixes (continued)

4. **Add RNA / template DNA** (>1ng - <1µg/reaction) to the individual PCR tubes.

5. **NOTE:** HotScriptase RT is amplifying DNA and RNA templates. If RNA detection or RNA quantification is desired, please use DNA-free RNA templates or use RNA-selective primers, which are binding onto exon-exon junctions!

6. **When using a thermal cycler with a heated lid**, do not use mineral oil. Proceed directly to step 6. Otherwise, overlay with approximately 50µL mineral oil.

7. **Program the thermal cycler** according to the manufacturer's instructions.
   A typical RT-PCR cycling program is outlined in Table 2. For maximum yield and specificity, temperatures and cycling times should be optimized for each new target or primer pair.

8. **Place PCR tubes in the thermal cycler and start program.**

Table 1: Recommendations for PCR / Reaction Setup (25µL PCR reaction)

Components	Volume	Final concentration
HotScriptase RT mastermix	12.5µL	1X
Primer forward (10µM)	1.25µL	0.5µM (0.05-1µM)
Primer reverse (10µM)	1.25µL	0.5µM (0.05-1µM)
Template/Sample extract*	xµL	>1ng (1-1000ng)
Nuclease-free water	up to 25µL total reaction volume	

*Recommended final template concentration is between 0.1ng/µL to 1ng/µL (total RNA).

Table 2: Typical RT-PCR protocol (an isothermal reverse transcription step is not needed)

Step	Temperature	Time
Initial denaturation	95°C	2 min.
Denaturation	95°C	15 sec.
Annealing/Extension*	various	45 sec. (25-40 cycles)
Hold	<10°C	hold

NOTE: HotScriptase RT does not have an activity optimum at 68°C as Taq-Polymerase. For this reason you can use much higher temperatures for elongation if needed (if the primer sequence does need higher temperatures)

***NOTE:** It is highly recommended to establish a new RT-PCR by running a temperature gradient in order to find the best annealing/extension temperature for each new primer pair!

A two-step as well as three-step PCR protocol can be used.
The annealing temperature of a primer is strongly influenced by its nucleic acid sequence and the reaction buffer composition (salts and pH). HotScriptase RT Polymerase is most active between 50-95°C.