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GENAXXON bioscience

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HotScriptase RT

Master mixes

Hotstart reverse Transcriptase and DNA Polymerase

**Master mix for parallel
reverse transcription and PCR (realtime PCR)
without isothermal transcription step.**

Cat#: M3062 (with Green Dye without ROX)
Cat#: M3064 (without Green Dye without ROX)

Version: 220818

GENAXXON bioscience GmbH
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- Molecular Biology Products
- PCR
- Proteins and Enzymes
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Related Products

	Contents	Cat. No.
M-MuLV Reverse Transcriptase	10000 units 50000 units	M3042.1010 M3042.5010
HotScriptase RT Polymerase for RT-PCR	250 units 1000 units	M3056.0250 M3056.1000
HotScriptase RT Mastermix Mastermix with GreenDye and with ROX for RT-PCR	1.25mL 5 x 1.25mL	M3057.0050 M3057.0250
HotScriptase RT Mastermix Mastermix with GreenDye for RT-PCR	1.25mL 5 x 1.25mL	M3062.0050 M3062.0250
HotScriptase RT Mastermix Mastermix without GreenDye with ROX for RT-PCR	1.25mL 5 x 1.25mL	M3063.0050 M3063.0250
HotScriptase RT Mastermix Mastermix w/o GreenDye for RT-PCR	1.25mL 5 x 1.25mL	M3064.0050 M3064.0250
HotScriptase RT Cell Mastermix Mastermix for RT-PCR from cells	1.25mL 5 x 1.25mL	M3058.0050 M3058.0250
Oligo dT primer lyophilized primer	5OD	M3039.0150
Random primer lyophilized primer	5OD	M3038.0125
dNTP-Set nucleotides for PCR	4 x 20µmol 4 x 25µmol 4 x 100µmol	M3015.4020 M3015.4025 M3015.4100
DNase from bovine pancreas	50mg 500mg	M3028.0050 M3028.0500
RNase A (free of DNase)	50mg 250mg	S5218.0050 S5218.0250
Total RNA Purification Mini Spin Kit	50 purifications 250 purifications	S5304.0050 S5304.0250

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Manual Contents

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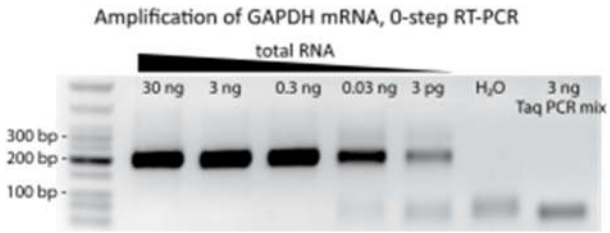
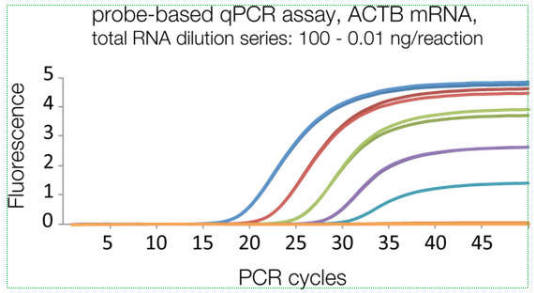
Application examples

"One-Step" RT-PCR: It can't be easier



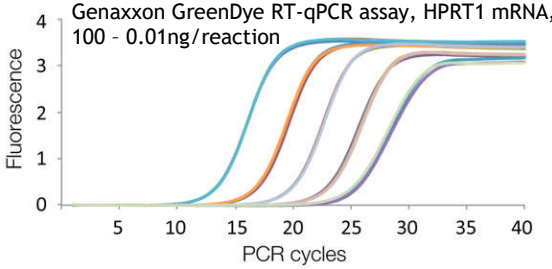
Amplification of GAPDH mRNA with HotScriptase RT is yielding the expected product amplicon length (194 bp), using various concentrations of RNA input. Negative Controls without total RNA or 3ng RNA but with *Taq* DNA polymerase (M3001). All products were run with the same zero-step RT-PCR protocol, analyzed on a 2.5% standard agarose gel.

Realtime PCR with HotScriptase RT: Probe-based qPCR



HotScriptase RT Master Mixes can also be used for realtime PCR. ACTB mRNA was detected using a 10-fold dilution series of total RNA by using a probe-based qPCR with Genaxxon HotScriptase RT Probe Master Mix and a "Zero-Step" RT-PCR protocol.

Realtime PCR with HotScriptase RT: GreenDye-based qPCR



HPRT1 mRNA was detected using a 10-fold dilution series of total RNA by using a GreenDye-based real-time qPCR assay and the zero-step RT-PCR protocol:Genaxxon HotScriptase RT Probe Master Mix and a "Zero-Step" RT-PCR protocol.

Quality Control

- RT-PCR activity: HotScriptase RT reverse transcriptase was tested for a successful RT-PCR performance.
  A 151 bp fragment (HPRT1 mRNA) was amplified from human total RNA extract in a PCR cycler and visualized as a single amplified product.
- DNA polymerase activity: HotScriptase RT activity has been monitored and adjusted to a specific DNA polymerase activity using an artificial DNA template and DNA primer.
- Enzyme-concentration has been determined by protein-specific staining. Please inquire more information at info@genaxxon.com for the lot-specific concentration.

Product Use Limitations

Genaxxon bioscience HotScriptase RT is developed, designed, and sold for research purposes only. It is not to be used for human, diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this manual.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please contact Genaxxon bioscience (info@genaxxon.com).

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Troubleshooting

Problem	Possible cause	Comments/suggestions
Negative control with *Taq* DNA polymerase is yielding a specific amplificate	Impurities in used reagents	Perform a reaction in the absence of any template, e.g. just MQ-water instead. If you are still getting a specific amplificate, you most likely have a contamination problem. Shortest solution is to start over with fresh samples and fresh reagents.
		Analyse your primer sequences and perform a primer BLAST to ensure, that your primers are only RNA-specific, for example here: http://www.ncbi.nlm.nih.gov/guide/howto/design-pcr-primers/. In case they are not RNA-specific, DNase digest your samples in order to remove any traces of DNA and try it again.
	Your are working with 16S DNA.	You have to use a complete DNA-free Polymerase.
Only getting primer dimers.		Keep all reagents during PCR setup on ice
		Program the PCR protocol on your cycler first, before you setup the PCR mix and if applicable preheat the lid of your cycler.
		When you do a new PCR for the first time, start with our standard protocol and recommended reaction setup concentrations.
		Run a temperature gradient to identify the best temperature yielding in the cleanest product. This temperature is only limited by the primer annealing temperature, as HotScriptase RT Polymerase is thermostable.- Use a "multiple primer analyzer" to test your primer sequences for self-complementarity, secondary structures and primer cross annealing

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HotScriptase RT master mix

Hotstart reverse Transcriptase and DNA Polymerase for RT-PCR

Product	Cat#	size
HotScriptase RT, 2-time Mastermix w/o Green Dye w/o ROX	M3064.0050	1.25mL
HotScriptase RT, 2-time Mastermix w/o Green Dye w/o ROX	M3064.0250	5 x 1.25mL
HotScriptase RT, 2-time Mastermix with Green Dye w/o ROX	M3062.0050	1.25mL
HotScriptase RT, 2-time Mastermix with Green Dye w/o ROX	M3062.0250	5 x 1.25mL

Product Description

The Genaxxon bioscience HotScriptase RT is an extremely thermostable enyzme that combines reverse Transcriptase and DNA Polymerase activity in one single enzyme.

HotScriptase RT facilitates “One-Step” RT-PCRs directly from RNA templates (without a separate isothermal reverse transcription step), as reverse transcription takes place simultaneously with DNA amplification during the cycled PCR elongation step. Besides the very convenient protocol this allows reverse transcription reactions at high temperatures, thus minimizing the problems encountered with strong secondary structures in RNA, or strong tertiary structures as in double strand RNA viruses that melt at elevated temperatures.

Genaxxon HotScriptase RT is **engineered and optimized for an amplicon size between 60- 400 bp** and is very well suited to be used for realtime PCR.

The very convenient HotScriptase RT 2-time master mixes contain all necessary ingredients for a successful and reliable RT-PCR with the exception of primers, probes or RNA/DNA. All HotScriptase RT 2-time master mixes can be used in all standard Thermal Cyclers. For the use in realtime instruments choose yourself if you do need master mixes with ROX or without or with a green fluorescence dye or without.

This product is for research use only

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Applications

- Rapid detection and identification of RNA targets
- Reverse transcription PCR (RT-PCR)
- realtime RT-PCR
- SELEX
- Primer extension
- Direct cell PCR

Product Specifications

Concentration:	2-time master mix
Half-life:	>40 min. at 95°C
5'-3' exonuclease activity:	Yes
Extra addition of A:	Yes
3'-5' exonuclease activity:	No
Nuclease contamination:	No
Protease contamination:	No
RNase contamination:	No
Self-priming activity:	No

Unit Definition

One unit of HotScriptase RT DNA-Polymerase is defined as the amount of enzyme that incorporates 10nmol of dNTP's into acid-insoluble fraction in 30 minutes at 72°C under standard assay conditions.

Stability

Genaxxon bioscience HotScriptase RT master mixes are shipped on wet ice.
Please store the product upon arrival at -20°C.

When stored under these conditions and handled correctly, the product can be kept at least until the expiration date (see tube label) without showing any reduction in performance.

FAQs

How can I design RNA-specific primers?

You can use one of the free primer design tools in the internet, such as primer-blast on this website: www.ncbi.nlm.nih.gov/tools/primer-blast/. Ensure, that you select the option: "primers must span an exon-exon junction". This is useful for limiting the amplification only to mRNA, as HotScriptase RT Poylmerase will amplify from any nucleic acid target consisting of both RNA or DNA.

Can I use HotScriptase RT Polymerase/Mastermix for use in realtime PCR?

Yes, our master mix is very well suited for realtime PCR respective reverse transcription + realtime PCR. For this you have to add fluorescent dye (e.g. SybrGreen) to your PCR reaction mixture or fluorogenic probes. Additionally, HotScriptase RT is much less sensitive to SybrGreen compared to other DNA polymerases. So you can use much higher concentrations of SybrGreen.

Note: We highly recommend to design primers with an annealing temperature >65°C!

Can I use HotScriptase RT in case my RNA shows very stable secondary structures and/or tertiary structures?

Yes, indeed, because you start with a 95°C denaturation step and you anneal above 60°C most likely, secondary or tertiary structures will be destroyed very effectively. For this HotScriptase RT Polymerase is very suitable in this case.

I am only getting primer dimers. How can I establish my RT-PCR?

- Keep all reagents during PCR setup on ice.

- Program the PCR protocol on your cycler first, before you setup the PCR mix and if applicable preheat the lid of your cycler.

- When you do a new PCR for the first time, start with our standard protocol and recommended reaction setup concentrations.

- Run a temperature gradient to identify the best temperature yielding in the cleanest product. This temperature is only limited by the primer annealing temperature, as HotScriptase RT Polymerase is thermostable.

- Use a "multiple primer analyzer" to test your primer sequences for self-complementarity, secondary structures and primer cross annealing.

Can I use the HotScriptase RT Cell master mix for procaryotic cells and yeasts?

HotScriptase RT Cell master mix is not established for use with procaryotic cells and yeasts yet. Due to very stable cell walls of these cells, you have to break them first to release the RNA or DNA. Then you have to treat the supernatant with DNase I because procaryots do not have exon-exon spans. This is necessary if you want to amplify only mRNA. As this is not a tested method, we recommend to isolate mRNA by established procedures (RNA isolation kits, e.g. our S5309) and use of our HotScriptase RT master mix M3057 for mRNA transcription and amplification.

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Alternative RT-PCR Protocol using HotScriptase RT master mixes

With short isothermal step before running the PCR program

If direct amplification of RNA is failing customers have been successful to implement a 5 minute isothermal step at 55-60°C before running the PCR program.

Table 3: Typical RT-PCR protocol (with isothermal reverse transcription step)

Use PCR reaction set up volumes shown on page 5

Step	Temperature	Time
Initial denaturation *	80°C	1 min.
isothermal step followed by one of the below mentioned PCR protocols	55°C - 60°C	5 min.

2-step PCR Protocol in case of TM >65°C		
Denaturation	95°C	10 sec.
Annealing/Extension*	>65°C - 70°C	45 sec. (25 - 40 cycles)
Alternatively use a 3-step PCR Protocol in case of TM >65°C		
Denaturation	95°C	10 sec.
Annealing	65°C - 70°C	30 sec.
Extension*	70°C - 75°C	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!

NOTE: Optimal PCR protocol times and temperatures may vary depending on the used cycler, the nature of template and the amplicon length.

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Important Notes

- **Amplicon length:** Amplicon length should not exceed 400bp.
  Genaxxon HotScriptase RT is engineered and optimized for an amplicon size between 60-400bp.
- **Primer:** Hotscriptase can only be used together with specific primers (forward and reverse). **Note: We highly recommend to design primers with an annealing temperature >65°C!**
- **Primer:** Use of intron spanning sequences, as HotScriptase RT polymerase will amplify RNA as well as DNA. Best will be to work with primers that are placed on an intron junction.
- **Annealing temperature** should be >65°C!
  The higher the annealing temperature, the better! 68°C are more suitable than 58°C.
- If you use HotScriptase RT the first time or if you establish a new primer set, please run a PCR with a temperature gradient at the annealing / extension step in order to find the optimal temperature for your assay respective each primer pair.
- The annealing temperature of a primer is strongly influenced by its nucleic acid sequence AND the reaction composition (pH, salts and salt concentration of used reaction buffer). It has been shown that the annealing temperature is most likely lower by about 2-3°C using the Genaxxon HotScriptase RT buffers compared to original systems!
- **Extension time:** Please start optimization with 60 sec. If you are fine with your results you may be able to reduce annealing time later.
- **Quantification of RNA/DNA:** Always run a PCR with a house-keeping gene target for being able to make calculations/judgements about the amount of your unknown sample.
- **qPCR:** Genaxxon HotScriptase RT can also be used for realtime cycling. Please note that Genaxxon HotScriptase RT is amplifying from both DNA and RNA templates. Use DNA-free RNA samples or use RNA-selective primers, which are binding onto intron junctions or which are exon-exon spanning.
- Genaxxon HotScriptase RT does not require Mn^{2+} for optimal reverse transcription activity. However, some assays may be further optimized by the addition of Mn^{2+} to the reaction (suggested range is 0.5 - 1.0 mM).
- **For initial optimization**: Use a short isothermal step of 7 - 10 minutes.

The No ROX master mixes (M3058 and M3064) are suited for example for following instruments:

Rotor-Gene™ / Corbet, DNA Engine Opticon™, Opticon® 2, Roche LightCycler®, Mastercycler® ep realplex 2, Smart Cycler®, Bio-Rad CFX96, 480Chromo 4™ Real-Time Detector.

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Protocol using HotScriptase RT Mastermix

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 additionally proceeding a "normal" PCR with *Taq* DNA polymerase 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 with probes.

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.**

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

Procedure using HotScriptase RT Mastermixes (continued)

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!

5. **When using a thermal cycler with a heated lid**, do not use mineral oil. Proceed directly to step 7. Otherwise, overlay with approximately 50µL mineral oil.

6. **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.

7. **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 master mix	12.5µL	1X
Primer forward (10µM)	1.25µL	0.4µM (0.05-1µM)
Primer reverse (10µM)	1.25µL	0.4µM (0.05-1µM)
Probe (10µM) (optional)	1 µL	0.3µM (0.05-1µM)
Template*/Sample extract**	x µL(not more than 9µL in case of sample extract)	variable volume: >0.1ng RNA or >250ng genomic DNA
Nuclease-free water	up to 25µL total reaction volume	

*Recommended final template concentration is between 0.1ng/µL to 1ng/µL (total RNA).
** 50 up to not more than 10000 cells/reaction

Table 2: Typical "One-Step" RT-PCR protocol without isothermal step

Step	Temperature	Time
Initial denaturation	95°C	3 min.
2-step PCR Protocol in case of TM >65°C		
Denaturation	95°C	15 sec.
Annealing/Extension*	>65°C - 70°C	60 sec. (25 - 40 cycles)
Alternatively use a 3-step PCR Protocol in case of TM >65°C		
Denaturation	95°C	15 sec.
Annealing	65°C - 70°C	30 sec.
Extension*	70°C - 75°C	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!

NOTE: Optimal PCR protocol times and temperatures may vary depending on the used cycler, the nature of template and the amplicon length.

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