MANUAL

Challenger Hotstart Reverse Transcriptase

Reverse transcriptase for one-step RT-qPCR/PCR with warm-start technology

Component	Cat#	M3068.1010	Colour code of cap
Challenger Hotstart Reverse Transcriptase (200U/µL)		1x 0.05mL	transparent
Challenger Hotstart RT 5X Buffer		1x 500µL	yellow

Description

The **Genaxxon Challenger Hotstart Reverse Transcriptase** is a thermostable, warm-start–enabled enzyme engineered for rapid, precise, and reliable cDNA synthesis. Its reversible aptamer-based modification keeps the enzyme completely **inactive during reaction setup** and activates it only at **55 °C**, ensuring full control over reaction initiation and **preventing nonspecific cDNA synthesis**.

By allowing convenient **room-temperature pipetting** without cooling steps, the enzyme streamlines workflows and minimizes the risk of errors. Its robust formulation maintains exceptional **stability** even in pre-assembled all-in-one reaction mixes, supporting reproducible performance under demanding storage and transport conditions.

The Challenger Hotstart Reverse Transcriptase combines **speed, sensitivity, and stability** for high-performance reverse transcription workflows. **Ultra-fast cDNA synthesis** can be achieved in as little as **2 minutes** without compromising yield or accuracy. More than 99 % of enzyme activity remains sealed at 42 °C and is only released upon activation at 55 °C, providing precise **temperature control** and **preventing premature reactions**. Even after **7 days at 37 °C** in premixed reaction setups, full enzymatic performance is retained, ensuring long-term stability and reliability.

Product specifications

Concentration:	200 U/µL
warm-start Technology	Yes
Nuclease contamination	No
Protease contamination	No
***E. coli* DNA**	< 1 copy

Advantages

Ultra-fast cDNA synthesis – as quick as 2 minutes
Warm-start control – prevents nonspecific cDNA synthesis at low temperatures
Extended stability – retains full performance after 7 days at 37 °C in premixed setups
High thermostability – > 99 % activity sealed at 42 °C, fully activated at 55 °C
Room-temperature setup – simplifies workflows and minimizes handling errors
Robust performance – ideal for routine, diagnostic, and high-throughput applications

Shipment: on wet ice

Long-Term Storage: Store at -20°C

Shelf-life: Stable until expiration date when stored and handled correctly

Unit definition

One unit of the Challenger Hotstart Reverse Transcriptase incorporates 1 nmol of dTTP into acid-precipitable material in 10 minutes at 37°C using poly(A)•oligo(dT) as template-primer.

Important notes before getting started

- This protocol serves as a guideline; for maximum efficiency and specificity, optimization may be required.
- Always use RNase-free consumables to prevent RNA degradation.
- Do not vortex enzyme; invert gently 6–8 times to mix. Excessive agitation may damage enzymes.

Reaction Setup Protocol

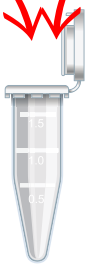
6-8 times

Thaw Challenger Hotstart Reverse Transcriptase and 5X Buffer at room-temperature or on ice.

Invert tube 6-8 times.

Do not vortex as this may harm the enzymes

Template RNA
Oligo(dT)18/Random Primer Mix
dNTP Mix
RNase Inhibitor
5X Buffer
Nuclease-free water



The recommended reaction volume is 20 µL.

Reaction setup

Components	Volume
Template RNA*	optional*
Oligo(dT)18 (50 µM) or Random Primer Mix (50 ng/µl)	1 µL
dNTP Mix (10 mM each)	1 µL
RNase Inhibitor (40 U/µL)	1 µL
Challenger Hotstart Reverse Transcriptase (200U/µL)	1 µL
Challenger Hotstart RT 5X Buffer	4 µL
Nuclease-free water	up to 20 µL

*Total RNA: 10pg - 5µg; Poly(A) RNA: 10pg - 500ng

Incubate the reaction mixtures as mentioned in the table on the right.

Standard Reaction Program

Temperature	Time
50 - 55°C	5 - 30min
85°C	2 min

The cDNA product can be stored at –25 °C to –15 °C or at –80 °C, or used immediately in PCR/qPCR reactions.
It is recommended that the volume of cDNA used in a PCR/qPCR reaction does not exceed one tenth (1/10) of the total reaction volume.

Additional information

Quality Control

Protein Purity (SDS-PAGE):	The enzyme is ≥ 98% pure as determined by SDS-PAGE analysis.
Endouclease activity:	Incubation of 200U of enzyme with 1 µg λ DNA for 16 hours at 37°C resulted in no detectable degradation of the DNA as determined by gel electrophoresis
Exonuclease activity:	Incubation of 200 U of enzyme with 1 µg λ-Hind III digest DNA for 16 hours at 37°C resulted in no detectable degradation of the DNA as determined by gel electrophoresis
Nickase Activity:	Incubation of 200 U of enzyme with 1 µg pBR322 for 16 hours at 37°C resulted in no detectable degradation of the DNA as determined by gel electrophoresis.
RNase Activity:	200 U of the enzyme with 1.6 µg MS2 RNA for 16 hours at 37°C yields no degradation as determined by agarose gel electrophoresis.
***E.coli* DNA:**	200 U of the enzyme is detected by TaqMan qPCR. The *E.coli* DNA is < 1 copy

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online as pdf-file or on request (info@genaxxon.com).

Product Use Limitations

Challenger Hotstart Reverse Transcriptase 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.

Further information

For more information, please visit our website: www.genaxxon.com
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