Manual

EpiScript RT PLUS

For Laboratory Use.

BIOSEARCH™ TECHNOLOGIES

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Contents

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1. Intended use

EpiScript™ Reverse Transcriptase PLUS (EpiScript RT PLUS) is a recombinant MMLV reverse transcriptase variant with greatly reduced RNase H activity. It is active up to 55 °C to achieve higher specificity and is efficient at producing both short and full-length cDNA from long RNA templates.

EpiScript RT PLUS is intended for use as a general purpose reagent in molecular diagnostic assays that are based on RT-PCR target detection technologies by clinical laboratory professionals.

This product is classified as a general purpose in vitro diagnostic device reagent in the United States as defined in 21CFR 864.4010.

2. Product description

Unlike other reverse transcriptases, EpiScript RT PLUS has significantly decreased polymerase activity at 25 °C due to a blocking mechanism, which enables room temperature reaction setup, and thus improved target specificity in RT-qPCR reactions. When paired with a hot start DNA polymerase, such as RapiDxFire Hot start Taq DNA Polymerase, a fully assembled reaction mix can be stored at room temperature until use improving overall relative fluorescence signals generated by high-throughput and automated RT-PCR applications. EpiScript RT PLUS is fully active at standard EpiScript RT reaction temperatures of 42 °C to 50 °C.

EpiScript RT PLUS is supplied at a concentration of 100 U/µL.

3. Warnings and limitations

For Laboratory Use. For Professional Use.

Outside the United States, FLU is the equivalent of Research Use Only labeling.

This product must be qualified and validated by clinical laboratory end-users for suitability in the detection of any specific target using the procedures in this Manual as guidance.

4. Product designations

Product	Size	Catalog number	Reagent description	Fill part number	Volume
EpiScript RT PLUS	12,500 Units	ERTPLUS-1	EpiScript RT PLUS, 125 µL @ 100 U/µL	F836566-5	125 µL
	125,000 Units	ERTPLUS-2	EpiScript RT PLUS, 1250 µL @ 100 U/µL	F836566-6	1250 µL
	1,250,000 Units	ERTPLUS-3	EpiScript RT PLUS 12.5 mL @ 100 U/µL	F836566-7	12.5 mL

5. Product storage and specifications

Storage: Store at -20 °C in a freezer without a defrost cycle.

Storage buffer: EpiScript RT PLUS is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM sodium chloride, 1 mM DTT, 0.1 mM EDTA, and 0.1% Triton X-100.

Unit definition: One unit of EpiScript RT PLUS catalyzes the incorporation of 1 nmol of dTTP into acid-insoluble material in 10 minutes at 37 °C using saturating amounts of oligo(dT)-primed poly(rA) as template.

Contaminating activity assays: EpiScript RT PLUS is free of detectable Endonuclease, Exonuclease, and RNase activity.

Purity: EpiScript RT PLUS is assessed as ≥95% pure by SDS-Polyacrylamide gel electrophoresis.

6. Materials supplied by the user:

- DNA polymerase (RapiDxFire Hot Start Taq DNA Polymerase, Cat # 30042, 30044)[1]
- Reaction Buffer (supplied with DNA polymerase)[1]
- Magnesium Chloride (25 mM supplied with DNA polymerase)[1]
- dNTP mix (25 mM each)[1]
- Target specific Primers/Assays
- RNA Template
- ROX solution (optional)
- RNase inhibitor, RiboGuard RNase Inhibitor Cat # RG90925 (optional)

[1] A PCR Master Mix can be utilised in place of individual components (RapiDxFire qPCR 5X Master Mix GF, Cat #30050).

7. Before you start:

Reaction preparation and handling

- Always thaw and mix reagents before use.
- EpiScript RT reactions may be assembled at room temperature when used alone or in combination with an appropriate hot-start DNA Polymerase.
- Assemble reactions on ice or in a cooling rack if using EpiScript RT PLUS with any component(s) that are sensitive to temperature.

Preparing templates

- RNA should be free of RNase contamination and aseptic conditions should be maintained.
- An RNase inhibitor such as RiboGuard (Cat # RG90925) may be added to the reaction mix to protect against ribonuclease contamination.

8. Protocol for one-step RT-qPCR

The protocol provides volumes for a single standard 20 µL reaction. For alternate reaction sizes, scale component volumes proportionally. To prepare a one-step RT-qPCR reaction with individual components, multiply each component volume by the number of desired reactions. Include an extra 10% volume to account for pipetting.

1. Thaw all reaction components completely, then vortex briefly and centrifuge to collect contents at the bottom of the tubes. Keep tubes on ice or cooling block at all times when not in use.
2. Combine the components in a sterile, nuclease free tube on ice in the order listed in the table below.

Component	Volume	Final concentration
Nuclease-free water[1]	to 20 µL	N/A
EpiScript RT PLUS	0.5 µL	2.5 U/µL
5 U/µL RapiDxFire Hot Start Taq DNA Polymerase	0.8 µL	0.2 U/µL
Hot Start Taq buffer	2 µL	1X
25 mM dNTP mix	0.2 µL	0.25 mM
25 mM MgCl2	3.2 µL	4 mM
10 µM Forward primer[2]	0.6 µL	0.3 µM
10 µM Reverse primer[2]	0.6 µL	0.3 µM
10 µM Probe[2]	0.4 µL	0.2 µM
40 U/µL Riboguard RNase Inhibitor (optional)	0.5 µL	1 U/µL
25 µM ROX (optional)[3]	X µL	50 nM – Low ROX Instrument 500 nM – High ROX Instrument
Template RNA[1]	X µL	N/A

[1] The amount of nuclease free water should be adjusted based on the volume of RNA Template and the optional addition of either RNase Inhibitor or ROX.
[2] Alternatively, a 20X assay mix containing the necessary target specific oligos can be used at a 1X final concentration.
[3] A passive reference dye such as ROX can be used for normalisation. The concentration of the passive reference dye should be chosen based on the instrument being used.

To prepare a one-step RT-qPCR reaction with a pre-existing qPCR Master Mix, add the following reagents in order.

Component	Volume	Final concentration
Nuclease-free water[1]	to 20 µL	N/A
EpiScript RT PLUS	0.5 µL	2.5 U/µL
RapiDxFire qPCR 5X Master Mix	4 µL	1X
Forward primer[2]	0.6 µL	0.3 µM
Reverse primer[2]	0.6 µL	0.3 µM
Probe[2]	0.4 µL	0.2 µM
40 U/µL Riboguard RNase Inhibitor (optional)	0.5 µL	1 U/µL
25 µM ROX (optional)[3]	X µL	50 nM – Low ROX Instrument 500 nM – High ROX Instrument
Template RNA	X µL	N/A

[1] The amount of nuclease free water should be adjusted based on the volume of RNA Template and the optional addition of either RNase Inhibitor or ROX.
[2] Alternatively, a 20X assay mix containing the necessary target specific oligos can be used at a 1X final concentration.
[3] A passive reference dye such as ROX can be used for normalisation. The concentration of the passive reference dye should be chosen based on the instrument being used. Alternatively, a master mix containing the appropriate concentration of passive reference dye can be utilised (RapiDxFire qPCR 5X Master Mix with UNG and ROX)

3. Perform RT-qPCR/RT-PCR using standard PCR cycling protocols

   The following thermal cycling protocol is for guidance only, for assays designed under standard conditions, using good-quality RNA. When working with non-standard assay design or with more complex target sequences, further optimisation may be required including an additional annealing step or a different extension temperature.

Step	Temperature	Time	Number of cycles
1. RT activation and reverse transcription	50 °C	15 minutes	1 cycle
2. Hot start Taq activation	95 °C	2 minutes	1 cycle
3. Template denaturation	95 °C	3 seconds	45 cycles
4. Primer annealing and extension	60 °C	30 seconds	

9. Further support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com

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