Lucigen
Reagent Components

# Lyo-Ready EpiScript Thermostable Reverse Transcriptase

LGC Diagnostics & Genomics

For laboratory use.

## Contents

# 1. Intended use

Lyo-Ready EpiScript™ Thermostable Reverse Transcriptase is a recombinant MMLV reverse transcriptase variant with greatly reduced RNase H activity and improved thermostability. It can be used for first-strand cDNA at temperatures up to 60°C to achieve higher specificity and can be paired with a qPCR master mix in one step RT-qPCR.

Lyo-Ready EpiScript Thermostable Reverse Transcriptase 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 located in the United States. It is glycerol-free and formulated to be compatible with lyophilisation. 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. This product is classified as a general purpose in vitro diagnostic device reagent in the United States as defined in: https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?FR=864.4010

Lyo-Ready EpiScript Thermostable Reverse Transcriptase is supplied at a concentration of 200 U/µL.

# 2. Product designations

| Product | Size | Catalog number | Reagent description | Fill part number | Volume |
|---|---|---|---|---|---|
| Lyo-Ready EpiScript Thermostable Reverse Transcriptase | 25,000 Units | 30260-1 | Lyo-Ready EpiScript Thermostable Reverse Transcriptase, 200 U/µL, 25,000 U, 0.125 mL | F867875-1 | 125 µL |

# 3. Product storage and specifications

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

**Storage buffer:** Lyo-Ready EpiScript Thermostable Reverse Transcriptase is supplied in a solution containing 20 mM Citrate (pH 6.4), 0.1 mM EDTA, 1 mM DTT, 0.05% Tween-20, 100 mM NaCl, 15% Trehalose.

**Unit definition:** One unit of Lyo-ready EpiScript Thermostable Reverse Transcriptase catalyses 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:** Lyo-ready EpiScript Thermostable Reverse Transcriptase is free of detectable endonuclease, exonuclease, and RNase activity.

**Purity:** Lyo-ready EpiScript Thermostable Reverse Transcriptase is assessed as ≥95% pure by SDS-Polyacrylamide gel electrophoresis.

## 4. Materials supplied by the user

- qPCR Master Mix (**RapiDxFire qPCR 5X Master Mix GF, Cat #30050, RapiDxFire qPCR 5X Master Mix with UNG, Cat #30051**, or **RapiDxFire qPCR 5X Master Mix with UNG and ROX, Cat # 30053**)
- Target specific primers/assays
- RNA template
- ROX solution (optional)
- RNase inhibitor, **RiboGuard RNase Inhibitor Cat # RG90925** (optional)

## 5. Before you start

### 5.1 Reaction preparation and handling

- Always thaw and mix reagents before use
- Assemble reactions on ice or in a cooling rack

### 5.2 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.

## 6. 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. To prepare a one-step RT-qPCR reaction with a pre-existing qPCR master mix, add the reagents detailed in table 1, in the order listed.

| Component | Volume | Final concentration |
|---|---|---|
| Nuclease-free water (1) | to 20 µL | N/A |
| Lyo-ready EpiScript Thermostable Reverse Transcriptase (2) | 0.5 µL | 5 U/µL |
| qPCR 5X Master Mix | 4 µL | 1X |
| Forward primer (3) | 0.6 µL | 0.3 µM |
| Reverse primer (3) | 0.6 µL | 0.3 µM |
| Probe (3) | 0.4 µL | 0.2 µM |
| 40 U/µL Riboguard RNase Inhibitor (optional) | 0.5 µL | 1 U/µL |
| 25 µM ROX (optional) (4) | X µL | 50 nM – Low ROX instrument<br>500 nM-High ROX instrument |
| Template RNA | X µL | N/A |

Table 1. Reagents required for a one-step RT-qPCR reaction.
(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) Units of Lyo-ready EpiScript Thermostable Reverse Transcriptase per reaction should be optimised and can be dependent on assay design and template quantity.
(3) Alternatively, a 20X assay mix containing the necessary target specific oligos can be used at a 1X final concentration. Final concentration should be optimised and can be dependent on assay design and template quantity.
(4) 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, Cat# 30053).

3. Perform RT-qPCR/RT-PCR using standard PCR cycling protocols. The thermal cycling protocol in table 2 is for guidance only, for assays designed under standard conditions, when 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 – Reverse transcription | 42 °C-55 °C (1) | 5-15 minutes (1) | 1 cycle |
| 2 – Hot start Taq activation | 95 °C | 2 minutes | 1 cycle |
| 3 – Template Denaturation | 95 °C | 10 seconds | 45 cycles |
| 4 – Primer annealing & extension | 60 °C | 30 seconds | |

Table 2. Standard thermal cycling protocol.
(1) The temperature and time for reverse transcription should be optimised for each primer design.

# 7. Troubleshooting Section

| Problem | Possible cause | Recommended solutions |
|---|---|---|
| Sub-optimal amplification results | Poor RNA integrity and/or purity | Evaluate integrity and purity prior to reaction via electrophoresis or automated microfluidic device |
| | Low RNA quantity | Verify RNA quantity by UV spectrophotometry or fluorescence-based method |
| | Inappropriate temperature | Ensure reaction temperature and primer design compatibility. Ensure reaction temperature is within optimal range of EpiScript Lyo-ready EpiScript Thermostable Reverse Transcriptase (42 °C-55 °C) |
| | Primer design issue | Ensure primer sequence is as intended for target and that they are able to distinguish RNA from gDNA by running a no reverse transcriptase reaction control |
| | Incompatibility with chosen qPCR Master Mix | EpiScript Lyo-ready EpiScript Thermostable Reverse Transcriptase has been verified for use with RapiDxFire 5x qPCR Master Mix. Compatibility with other qPCR Master Mixes will have to independently verified |
| | Inhibitors present within sample | Ensure RNA is purified and that preparation removes carryover from RNA extraction technique |
| | RT concentration, incubation temperature, and reaction time should be optimized for each primer/template | RT concentration: 80-160 units per reaction<br>RT temperature: 37-60 °C<br>RT time: 1 min-15 min |
| Non-specific amplification | Sub-optimal primer design | Check primer designs for potential annealing to unintended targets |
| | Sub-optimal reaction temperature | Increase temperature to ensure stringency in primer-target interaction |

# 8. Further support

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

Visit LinkedIn | lgcgroup.com/what-we-do/lucigen-reagent-components

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