MeloScript Reverse Transcriptase

Cat. No.: FP1509103 | Pack size: 20 KU / 200 KU / 2000 KU | Storage: -20 °C

Overview

MeloScript Reverse Transcriptase is an engineered reverse transcriptase developed through in vitro molecular evolution technology. It is designed to resist common inhibitors present in RNA extracts, such as isopropanol residues and lysis-buffer components, enabling reliable performance with challenging or suboptimal samples.

The enzyme provides high-fidelity reverse transcription of difficult templates, including low-abundance, high-GC, or highly structured RNA, and improves target capture rate and downstream assay sensitivity in one-step Real-Time RT-PCR and first-strand cDNA synthesis workflows.

Key Features

- Engineered reverse transcriptase with enhanced inhibitor tolerance
- High-efficiency reverse transcription of low-abundance, high-GC, or structured RNA templates
- Suitable for one-step Real-Time RT-PCR and first-strand cDNA synthesis
- DNase/RNase-free EnzymoPure™ grade for molecular biology applications
- Supplied at 200 U/μL with 5× MeloScript Buffer

Product Specifications

Parameter	Specification	Notes
Product name	MeloScript Reverse Transcriptase	Engineered reverse transcriptase
Cat. No.	FP1509103	Available pack sizes: 20 KU, 200 KU, 2000 KU
Grade & purity	DNase, RNase free; Suitable for molecular biology; EnzymoPure™; for DNA and RNA applications	Supplied enzyme concentration: 200 U/μL
Bioactivity	200 U/μL	Reverse transcriptase activity
Application	One-step Real-Time RT-PCR; first-strand cDNA synthesis	Use assay-specific primers or suitable RT primers
Storage conditions	Store at -20 °C; avoid repeated freezing and thawing	Aliquot upon receipt when repeated use is expected

Parameter	Specification	Notes
Shipping	Ice chest + ice pads	Store at -20 °C immediately upon receipt
Stability	Store at -20 °C long term (12 months)	Avoid freeze/thaw cycles

Product Contents & Storage

Cat. No.	Component	20 KU	200 KU	2000 KU	Storage
FP1509103A	MeloScript Reverse Transcriptase (200 U/μL)	100 μL	1.0 mL	10 mL	-20 °C
FP1509103B	5× MeloScript Buffer	1.0 mL	10 × 1.0 mL	100 mL	-20 °C

Shelf life: Stable for 12 months when stored at -20 °C under recommended conditions.

Note: *Avoid repeated freeze/thaw cycles. Keep the enzyme on ice during use and return it to -20 °C immediately after use.*

Materials Required But Not Supplied

Item	Recommended Specification	Purpose
Template RNA	Purified total RNA or target RNA sample	Reverse transcription template
dNTP mix	25 mM each	DNA synthesis substrates
Forward primer / Reverse primer	Gene-specific primers, typically 10 μM working solutions	Target-specific amplification
Probe	10 μM working solution, assay-specific	Real-Time PCR detection
RNase inhibitor	40 U/μL or equivalent	Protection from RNase contamination
PowerResist Taq Polymerase	5 U/μL or validated hot-start Taq polymerase	PCR amplification in one-step RT-qPCR
Nuclease-free H_2O	RNase/DNase-free	Reaction volume adjustment

Item	Recommended Specification	Purpose
RNase-free tubes, tips, and pipettes	Certified nuclease-free consumables	Contamination control
Real-time PCR instrument	Compatible with probe-based RT-qPCR assays	Thermal cycling and fluorescence acquisition

Preparation Before Use

1. Thaw 5× MeloScript Buffer and other frozen reagents at room temperature or 4 °C. Mix thoroughly and briefly centrifuge before use.
2. Keep MeloScript Reverse Transcriptase and prepared reaction mixtures on ice during operation.
3. Use only RNase-free tubes, pipette tips, and nuclease-free water. Prepare reactions in a clean area dedicated to RNA work when possible.
4. Aliquot enzyme and buffer upon receipt to minimize freeze/thaw cycles.

Protocol

Step 1 — Reaction Setup (25 µL System)

Mix gently after reaction assembly and briefly centrifuge. Avoid vigorous vortexing of enzyme-containing mixtures.

Component	Volume	Final Concentration
5× MeloScript Buffer	5 µL	1×
dNTP (25 mM each)	0.2 µL	0.2 mM
Forward Primer (10 µM)	0.5 µL	0.2 µM
Reverse Primer (10 µM)	0.5 µL	0.2 µM
Probe (10 µM)	0.25 µL	0.1 µM
RNase Inhibitor (40 U/µL)	0.25 µL	0.4 U/µL
MeloScript Reverse Transcriptase (200 U/µL)	0.2 µL	1.6 U/µL
PowerResist Taq Polymerase (5 U/µL)	0.5 µL	0.1 U/µL
Template RNA	5 µL	-
Nuclease-free H_2O	To 25 µL	-
Total	25 µL	-

Step 2 — Thermal Cycling

Step	Temperature	Time	Cycles
Reverse Transcription	50 ℃	15 min	1
Initial Denaturation	95 ℃	2 min 30 sec	1
Denaturation	95 ℃	15 sec	35-40
Annealing/Extension	55 ℃	20 sec	35-40

Step 3 — Optimization Tips

- For structured or high-GC templates, increase RT temperature to 55 ℃ after validation.
- For rapid protocols, RT time may be reduced to 5 min after assay validation.
- Adjust primer concentration within 0.1-1.0 μM and probe concentration within 50-250 nM as needed.
- Use amplicons of 80-200 bp for optimal qPCR performance.
- Adjust data acquisition time according to instrument requirements: ABI 7700/7900HT ≥30 sec; ABI 7000/7300 ≥31 sec; ABI 7500 ≥34 sec.

Step 4 — Primer Selection Guidelines

- For eukaryotic RNA in PCR workflows, Oligo(dT) may be used for full-length cDNA synthesis.
- For prokaryotic RNA or structured regions, random hexamers may improve coverage.
- For target-specific reverse transcription or one-/two-step RT-qPCR, use gene-specific primers (GSP).
- Prefer G/C at primer 3'-ends; avoid hairpins, consecutive mismatches near the 3'-end, and primer-dimers.

Unit Definition

One unit (U) is defined as the amount of enzyme required to incorporate 1 nmol of dTTP into acid-insoluble material in 10 min at 37 ℃ using poly(rA)·oligo(dT) as template/primer.

Enzyme Handling & Precautions

- Maintain a clean workspace with RNase-free consumables. Wear gloves and masks during handling.
- Avoid vortexing the reverse transcriptase; mix gently and keep enzyme on ice during use.

- Excess RT enzyme may cause nonspecific PCR products or reduced plateau fluorescence. Use the recommended amount as a starting point.
- Read the current SDS before use and handle reagents according to laboratory safety procedures.

Quality Control

QC Item	Method	Acceptable Range
Component completeness	Visual inspection on receipt	Components, labels, pack size, lot number, and expiry information match the product contents table
Enzyme concentration	Approved product specification and activity assignment	MeloScript Reverse Transcriptase supplied at 200 U/µL
Functional performance	PCR/qPCR amplification with qualified template	Expected amplification in positive reactions; no amplification in appropriate negative controls
Nuclease status	DNase/RNase functional testing where applicable	Meets approved specification for molecular biology applications
Storage verification	Review receipt condition and storage record	Cold-chain shipment received intact and stored at -20 °C immediately

Troubleshooting

Issue	Possible Causes	Corrective Action
No or weak amplification	• RNA template degraded or contains inhibitors • Missing reaction component • Reverse transcription temperature/time not optimal • Enzyme activity reduced by repeated freeze/thaw	• Check RNA integrity and purity; dilute inhibitor-containing templates • Verify all components and reaction volumes • Optimize RT temperature/time within the recommended range • Use freshly thawed aliquots and keep enzyme on ice

Issue	Possible Causes	Corrective Action
Nonspecific amplification or primer-dimer	• Primer design is not optimal • Primer/probe or enzyme concentration too high • Annealing/extension temperature too low	• Redesign primers and verify specificity • Reduce primer/probe concentration after validation • Optimize annealing/extension temperature and cycling conditions
Poor reproducibility or high Ct variation	• Inaccurate template pipetting • Inconsistent mixing • Variable RNA input	• Dilute templates to 2-5 µL/sample for easier pipetting • Prepare master mix on ice and mix gently but thoroughly • Run technical replicates and normalize RNA input
Signal in NTC or no-RT controls	• Reaction setup contamination • Genomic DNA carryover	• Prepare reactions in a clean area using nuclease-free consumables • Include no-template and no-RT controls • Treat RNA with DNase if genomic DNA interference is suspected

Recommended Applications

One-step Real-Time RT-PCR · first-strand cDNA synthesis · RT-qPCR assay development · analysis of inhibitor-containing or structured RNA samples

Contact & Global Offices

Whether you have a technical question, need help with a quotation, or want to inquire about an order, our regional teams are ready to assist. Please contact the office for your region; for general inquiries, the North American office is the corporate primary.

NORTH AMERICAN SALES, SUPPORT & GENERAL INQUIRIES

Aladdin Scientific Corporation

14078 Meridian Parkway, Riverside, CA 92518, USA

Phone: 1-833-552-7181

Sales: sales@aladdinsci.com

Customer Service: custserv@aladdinsci.com

EU SALES, LOGISTICS & LOCALIZED SUPPORT

Aladdin Biochem Deutschland GmbH

Westring 2, 33142 Büren, Germany

Phone: +02951 9383958

Support: support.eu@aladdinsci.com

Limitations & Disclaimer

- For Research Use Only (RUO). Not for use in human or animal diagnostics, therapeutics, or in vivo applications. Not for food, cosmetic, or household use.
- This product is not a CE-marked in vitro diagnostic device under IVDR (EU) 2017/746 and is not an FDA-cleared device under 21 CFR. Use is restricted to verified businesses, institutions, and qualified professionals for research and development purposes.
- Where any component is classified as hazardous under CLP (EC) 1272/2008 or OSHA HCS (29 CFR 1910.1200), the product Safety Data Sheet (SDS) takes precedence over this document for handling, storage, and disposal information.
- Performance depends on sample quality, assay design, reaction setup, instrument settings, and operator technique. Users are responsible for validating the product for their specific application.
- Aladdin product labels, SDS, COA, and approved specifications take precedence over this document. If product formulation, label, SDS, storage conditions, pack size, or quality specifications change, this document would be reviewed and reissued.