

User Guide

Heat-labile Cod Uracil-DNA Glycosylase

Recombinant, from E. coli

SRE0111 (Triton-free)

SRE0112 (Triton-free, Glycerol-free)

Product Overview

Description

Heat-labile Cod Uracil-DNA Glycosylase (UNG or UDG) prevents the carryover of unwanted DNA contaminants (e.g. from previous PCRs) through cleavage of uracil incorporated into the phosphodiester backbone of DNA amplified using dUTP in the dNTP mix.¹ The enzyme undergoes irreversible deactivation at 55 °C, making it compatible with one step RT-qPCR experiments and any application where evaluation of stored DNA product post-PCR is necessary.

Heat-labile Cod UNG is supplied at 1 U/ μ L (SRE0111) or 50 U/ μ L (SRE0112). The Atlantic Cod enzyme is expressed recombinantly in a *ung*- strain of *E. coli* to eliminate contamination with thermostable versions of the enzyme.

Features

- Heat-labile
- Compatible with JumpStart™ Taq DNA Polymerase
- Compatible with one-step RT-qPCR
- Triton (alkylphenol ethyoxylate)-free
- Glycerol-free (SRE0112 only)

Applications

- PCR and qPCR
- RT-PCR and RT-qPCR
- LAMP and RT-LAMP

Unit Definition

One unit of UNG is defined as the amount of enzyme required to release 1 nmol uracil from uracil-containing DNA per hour at 37 °C in 70 mM Tris-HCl, 10 mM NaCl, 1 mM EDTA and 0.1 mg/ml BSA.

Reagents Provided

Heat-labile Cod Uracil-DNA Glycosylase

- SRE0111, 1 U/μL
- SRE0112, 50 U/µL glycerol-free

Materials and Reagents Required (not included)

- PCR product amplified with dUTP
- Nuclease-free PCR tubes
- Thermocycler or incubator

Precautions and Disclaimer

This product is for R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at -20 °C.

Suggested Protocols

PCR Carryover Elimination

- 1. Add 0.2 U UNG to a 20 μL PCR product generated with dUTP
- 2. Pre-incubate 5 minutes at room temperature Note: the pre-incubation step can be skipped if UNG amount is increased to 1 U per 20 μ L PCR product generated with dUTP
- 3. Run PCR

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RT-qPCR Contamination Cleanup

- 1. Add 0.2 U of UNG to a 20 μ L PCR product generated with dUTP
- 2. Pre-incubate 5 minutes at room temperature Note: the pre-incubation step can be skipped if UNG amount is increased to 1 U per 20 μ L PCR product generated with dUTP
- 3. Reverse transcribe RNA at 50 55 °C if using a thermostable polymerase; incubate at 55 °C for 5 minutes and then reverse transcribe RNA at manufacturer-recommended temperature if using MMLV or other non-thermostable polymerase.
- 4. Run PCR

RT-LAMP or LAMP

1. Add 1 U UNG per 30 μ L RT-LAMP or LAMP reaction containing dUTP on ice

Note: Run a preliminary experiment to ensure RT-LAMP is compatible with dUTP by testing different dUTP:dTTP ratios in the dNTP mix side by side (e.g., 100% dUTP, 90% dUTP, 80% dUTP and 0% dUTP)

2. Run reaction at 65 °C

References

1. Longo MC, et al., Gene, 93,125-128 (1990).

Technical Assistance

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Contact Information

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Product Ordering

Order products at SigmaAldrich.com.

Description	Catalogue Number
Water, Microbial DNA-free	MBD0025
JumpStart™ Taq DNA Polymerase	D4184
M-MLV Reverse Transcriptase	M1302
Quantitative RT-PCR ReadyMix™	QR0200
Deoxynucleotide Set, 100 mM	DNTP100
2'-Deoxyadenosine 5'-triphosphate sodium salt solution (dATP)	D4788
2'-Deoxycytidine 5'-triphosphate disodium salt (dCTP-Na ₂)	D4913
2'-Deoxyguanosine 5'-triphosphate trisodium salt solution (dGTP-Na ₃)	D5038
2'-Deoxyuridine 5'-triphosphate sodium salt solution (dUTP sodium salt)	D0184
Nuclease-Free Water, for Molecular Biology	W4502

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