AmpErase® Uracil N-Glycosylase (UNG)

Package Contents

Catalog Number	Size	
N8080096	100 Units	Kit Contents

Storage Conditions

- Store at –20°C until just prior to use.

Required Materials

- Template: cDNA, gDNA, λDNA
- Forward and reverse gene-specific primers
- Autoclaved, distilled water
- E-Gel® General Purpose Gels, 1.2% (Cat. no. G5018-01)
- 0.2 or 0.5-mL nuclease-free microcentrifuge tubes
- 100-mM dUTP solution (Cat. no. R0133)

For PCR: GeneAmp® RNA PCR Core Kit (Cat. no. N8080143)

For qPCR:

- TaqMan® GAPDH Control Reagents (human) (Cat. no. 402869)
- TaqMan® Universal PCR Master Mix, No AmpErase® UNG (Cat. no. 4326614)
- 20X TaqMan® Primer/Probe Mix
- MicroAmp® EnduraPlate™ Optical 384-well Plate (Cat. no. 4483285)

Timing

Varies depending on amplicon length

Selection Guides

Go online to view related products.
PCR Enzymes and Master Mixes

Product Description

- AmpErase® Uracil N-Glycosylase (UNG) is a 26-kDa ultrapure, recombinant enzyme encoded by the *E. coli* uracil N-glycosylase gene, designed to degrade PCR products from previous amplifications or mis-primed, non-specific products, without degrading native nucleic acid templates.
- PCR products are made susceptible to degradation by substituting dUTP for dTTP in the reaction mix and testing subsequent mixes with the UNG prior to amplification.

Important Guidelines

- Take precautions to avoid cross-contamination by using aerosol-resistant barrier tips and analyzing PCR products in a separate area from PCR assembly.
- Because AmpErase® UNG has activity below 55°C, the annealing temperature should be ≥ 55°C to avoid degradation of newly synthesized dU-containing products by residual UNG activity.
- Dilute 100 mM dUTP to 20 mM prior to using in PCR reaction.
- **For qPCR applications, dilute UNG 1:5 in TE pH 8.0.**

Online Resources

Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support.

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Print Options

PCR Reaction Setup

Use the measurements below to prepare your PCR experiment, or enter your own parameters in the column provided.

Component	100-µL rxn	Custom	Final Conc.
Autoclaved, distilled water	to 100 µL	to µL	–
10X PCR Buffer II	10 µL	µL	1X
10 mM dATP	2.0 µL	µL	0.2 mM
10 mM dCTP	2.0 µL	µL	0.2 mM
10 mM dGTP	2.0 µL	µL	0.2 mM
20 mM dUTP*	1–5 µL	µL	0.2–1.0 mM
25 mM $MgCl_2$**	8.0 µL	µL	2.0 mM
10 µM Forward primer	2.0 µL	µL	0.2 µM
10 µM Reverse primer	2.0 µL	µL	0.2 µM
Template DNA	Varies		< 2.0 µg/100 µL
AmpliTaq® DNA Polymerase (5 U/µL)	0.5 µL	µL	2.5 U/100 µL
AmpErase® UNG (1 U/µL)	1 µL	µL	1 U/100 µL

* Dilute dUTP to 20 mM prior to using it in PCR reaction. Due to the lower efficiency of dUTP incorporation, using any amount of dUTP in the recommended range may result in lower amplification than using the standard dTTP concentration (0.2 mM/50-µL rxn).

** Increase $MgCl_2$ on an equimolar basis when an increased dUTP concentration is required. The magnesium ion is not required for UNG activity.

PCR and qPCR Protocols

See pages 2 and 3 to view procedures for preparing and running your PCR and qPCR experiments.

Optimization Strategies

Refer to the pop-ups below for guidelines to optimize your PCR reactions.

DUG Guidelines | PCR Guidelines | qPCR Guidelines

Limited Warranty, Disclaimer, and Licensing Information

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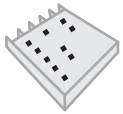
PCR Amplification Protocol

The example PCR procedure below shows appropriate volumes for a single **100-µL** reaction. For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, and then dispense appropriate volumes into each 0.2–0.5 mL PCR reaction tube prior to adding template DNA and primers.

Timeline	Steps	Procedure Details
1 	**Thaw reagents**	Thaw, mix, and briefly centrifuge each component before use. **Note:** Avoid generating bubbles when mixing the enzyme.
2 	**Prepare PCR master mix**	Add the following components to each PCR reaction tube. **Note:** Consider the volumes for all components listed in steps 2 and 3 to determine the correct amount of water required to reach your final reaction volume.
3 	**Add primers and template DNA**	Add the primers and template DNA to each tube for a final reaction volume of 100 µL.
4 	**Incubate reactions in a thermal cycler**	Cap, mix, and then centrifuge the contents.
5 	**Analyze with gel electrophoresis**	Analyze 10 µL using agarose gel electrophoresis. Use your PCR reaction immediately for down-stream applications, or store it at –20°C.

Component | 100-µL rxn | Final Concentration

Component	100-µL rxn	Final Concentration
Autoclaved, distilled water	to 100 µL	–
10X PCR Buffer II	10 µL	1X
10 mM dNTPs (dATP, dCTP, dGTP)	2 µL each	0.2 mM
20 mM dUTP*	2 µL	0.4 mM
25 mM $MgCl_2$**	8 µL	2.0 mM
AmpliTaq® DNA Polymerase (5 U/µL)	0.5 µL	2.5 U/100 µL
AmpErase® UNG (1 U/µL)	1 µL	1 U/100 µL

* Dilute 100 mM stock to 20 mM prior to use.
** Increase $MgCl_2$ on an equimolar basis, when an increased dUTP concentration is required. The magnesium ion is not required for UNG activity.

Mix and briefly centrifuge the components.

Component | 100-µL rxn | Final Concentration

Component	100-µL rxn	Final Concentration
10 µM forward primer	2 µL	0.2 µM
10 µM reverse primer	2 µL	0.2 µM
Template DNA	varies	< 2.0 µg/rxn

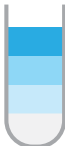
Step | Temperature | Time

Step		Temperature	Time
Pre-incubation		50°C	2 minutes
Initial Denaturation		95°C	2 minutes
35 PCR Cycles	Denature	95°C	15 seconds
	Anneal	~55°C (depending on Primer T_m)*	30 seconds
	Extend	37–65°C	1 minute/kb
Hold		72°C	7 minutes

* Adjust the temperature according to the primer melting temperature, but ensure it is at least 50°C.

qPCR Protocol

The example qPCR procedure below shows appropriate volumes for a single **20-µL** reaction. For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, and then dispense appropriate volumes into each 0.2–0.5 mL PCR reaction tube, or well of a MicroAmp® EnduraPlate™ Optical 384-well plate, prior to adding template DNA and primers.

Timeline	Steps	Procedure Details
1 	**Thaw reagents**	Thaw reagents. Set up reactions on ice. For 384-well plates, we recommend a maximum reaction volume of 10 µL per well. **Note:** Always prepare no-template control (NTC) reactions to test for DNA contamination of the enzyme/primer mixes. Use TaqMan® GAPDH control reagents to confirm reaction set-up.
2 	**Prepare qPCR master mix**	See table below.
3 	**Incubate reactions in a real-time instrument**	See table below.
4 	**Collect and analyze data**	Analyze results following your Real-Time instrument manufacturer's guidelines. **Optional:** The specificity of the PCR products can be checked by agarose gel electrophoresis.

Component	20-µL rxn	Final Concentration
Autoclaved, distilled water	to 20 µL	–
TaqMan® Universal PCR Master Mix, No AmpErase® UNG	10 µL	1X
AmpErase® UNG (1 U/µL)*	0.2 µL	0.04 U/20 µL
20X Fluorescent Primer/Probe Mix	1 µL	1X
cDNA (10 to 100 ng)	2 µL	varies

* Prepare a fresh 1:5 dilution in TE buffer prior to use. It may be necessary to optimize the dilution of UNG (1:5–1:10) for specific targets. We recommend a fresh 1:5 dilution as a starting point.
Note: Do not store or reuse diluted enzyme.

Cap or seal each PCR tube/plate, gently mix, and centrifuge contents.

Step		Temperature	Time
Pre-incubation		50°C	2 minutes
Heat activation		95°C	10 minutes
40 cycles	Amplification	95°C	15 seconds
		60°C	1 minute