

Uracil-N-glycosylase

For use with QuantiTect® PCR Kits to eliminate carryover of PCR products

Kit Contents

Uracil-N-glycosylase*	(100 U)
Number of 50 µl reactions	200
Uracil-N-glycosylase solution (1 U/µl)	100 µl

* Uracil-N-glycosylase is included in several QuantiTect PCR Kits (see ordering information, back page). It is not available separately.

Shipping and Storage

Uracil-N-glycosylase is shipped on dry ice. The product should be stored immediately upon receipt at -20°C in a constant-temperature freezer. When the product is stored under these conditions and handled correctly, performance is guaranteed until the expiration date (see the quality-control label on the zip-lock bag).

Product Description

Uracil-N-glycosylase is a pure, nuclease-free recombinant 26 kDa enzyme, expressed in *E. coli*.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/Support/MSDS.aspx where you can find, view, and print the MSDS for each QIAGEN® kit and kit component.

24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240



Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each component of uracil-N-glycosylase is tested against predetermined specifications to ensure consistent product quality.

Introduction

PCR products from previous PCR runs are a major source of PCR contamination, known as carryover contamination. This problem can be reduced by enzymatic destruction of contaminants. To enable this step to be performed, dTTP in QuantiTect PCR Kits is partially or completely substituted by dUTP. In addition, uracil-N-glycosylase (UNG) must be added to the PCR at an appropriate concentration (see procedure below). An incubation step of 50°C for 2 minutes is added to the cycling program, before activation of HotStarTaq® DNA Polymerase. During this incubation step, UNG removes uracil from dUMP incorporated into any contaminating molecules, leaving apyrimidinic sites. During the activation step of HotStarTaq DNA Polymerase (15 minutes at 95°C), the UNG is inactivated, and contaminating molecules are destroyed by hydrolysis of the phosphate backbone at the abasic sites. During subsequent cycling, only target nucleic acid will be amplified; contaminating molecules from previous reactions will not be amplified. QIAGEN UNG is specially developed for use in QuantiTect buffers to provide effective elimination of carryover contamination.

Note: Although QIAGEN UNG is inactivated by incubation at 95°C for 15 minutes, the enzyme may exhibit residual activity at lower temperatures due to refolding. We therefore recommend performing subsequent PCR using a temperature of 55°C or above for the annealing step.

Note: Do not use QIAGEN UNG in real-time one-step RT-PCR. The UNG will remain active during the reverse-transcription step and destroy any newly synthesized cDNA. **Heat-labile** UNG is required for real-time one-step RT-PCR.

Procedure

1. **Follow the relevant protocol in the handbook supplied with the QuantiTect PCR Kit you are using.**

2. **Add QIAGEN UNG when preparing the reaction mix.**

A 50 μ l reaction requires 0.5 units UNG (i.e., 0.5 μ l of QIAGEN UNG solution). A 25 μ l reaction requires 0.25 units UNG (i.e., 0.25 μ l of QIAGEN UNG solution).

3. **Include the UNG incubation step when programming your real-time cyclers.**

The UNG incubation step involves incubating reactions for 2 min at 50°C, and precedes the HotStarTaq DNA Polymerase activation step of 15 min at 95°C.

4. **After the reaction is completed, immediately store the PCR products at –20°C if they will be used in downstream applications such as agarose gel analysis.**

Although QIAGEN UNG is inactivated by incubation at 95°C for 15 min, the enzyme may exhibit residual activity at lower temperatures due to refolding. To avoid degradation of the PCR products, we recommend maintaining them at 72°C at the end of the reaction and then transferring them to frozen storage at –20°C as soon as possible. QIAGEN UNG exhibits no activity at temperatures above 72°C or below –20°C.

Ordering Information

Product	Contents	Cat. no.
For real-time PCR and two-step RT-PCR using SYBR® Green I		
QuantiTect SYBR Green PCR +UNG Kit (200)	For 200 x 50 µl reactions: 3 x 1.7 ml 2x QuantiTect SYBR Green PCR Master Mix, 100 µl UNG, 2 x 2 ml RNase-Free Water	204163
For real-time PCR and two-step RT-PCR using sequence-specific probes		
QuantiTect Probe PCR +UNG Kit (200)	For 200 x 50 µl reactions: 3 x 1.7 ml 2x QuantiTect Probe PCR Master Mix, 100 µl UNG, 2 x 2 ml RNase-Free Water	204363

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