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Instruction manual Uracil-DNA Glycosylase (UNG), Heat-labile 1907

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Uracil-DNA Glycosylase (UNG), Heat-labile

Code No. UNG-101　200 U×1
Store at −20°C

[1] Introduction

Uracil-DNA Glycosylase (UNG) hydrolyzes the N-glycosylic bond between the deoxyribose sugar and the base in uracil-containing DNA leaving an apyrimidinic site in DNA. Since DNA with an apyrimidinic site is degraded by heat treatment, UNG can be used in combination with dUTP to prevent carry-over of amplified products derived from PCR.

This enzyme hydrolyzes uracil from both single- and double-stranded DNA containing dU, but it not from RNA. It can also be used to prevent carryover of RT-PCR.

[2] Components

Product name	Conc.	Package	Code No.	Storage
Uracil-DNA Glycosylase (UNG), Heat-labile	1 U/μL	200 U×1	UNG-101	−20°C

[3] Properties

(1) 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.

(2) Heat inactivation

This enzyme is completely and irreversibly inactivated by incubation for 10 minutes at 50°C.

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[4] Source

Produced in *E. coli* (ung-) strain expressing a recombinant uracil DNA glycosylase gene of Atlantic cod (*Gadus morhua*) UNG.

[5] Buffer composition

Conc.	Component
20 mM	Tris-HCl, pH 7.5 (25℃)
50 mM	NaCl
1 mM	DTT
0.1% (v/v)	Tween20
50% (v/v)	Glycerol

[6] Usage example

By adding to PCR reagent and incubating at room temperature (20-25℃) just prior to PCR reaction (RT reaction in RT-PCR), it is possible to eliminate carryover of uracil-containing amplified products. Since UNG is inactivated during PCR denaturation, there is no effect on the following reaction.

The following table shows a preparation example and reaction cycling conditions using UNG and our qRT-PCR reagent (THUNDERBIRD™ Probe One-step qRT-PCR Kit, Code: QRZ-101). Please change the amount of UNG as necessary.

Component	20 µL scale	50 µL scale	Final conc.
RNase free water	X µL	X µL	
2 × Reaction Buffer	10 µL	25 µL	1 ×
DNA Polymerase	0.5 µL	1.25 µL	
RT Enzyme Mix	0.5 µL	1.25 µL	
Forward Primer	10 pmol	25 pmol	0.5 µM
Reverse Primer	10 pmol	25 pmol	0.5 µM
TaqMan® probe	4 pmol	10 pmol	0.2 µM
50 × ROX Reference dye	0.4 / 0.04 µL	1 / 0.1 µL	1 × / 0.1 ×
Uracil-DNA Glycosylase（UNG）, Heat-labile	**0.4 µL**	**1.0 µL**	**0.4 / 1.0 U**
RNA solution	Y µL	Y µL	
Total reaction volume	20 µL	50 µL	

Cycling conditions		Temperature	Time
UNG reaction		**25℃**	**10 min.**
Reverse transcription		50℃	10 min.
Denaturation		95℃	1 min.
PCR	Denaturation:	95℃	15 sec.
(40～45 cycles)	Extension (Annealing)	60℃	45 sec.

[7] Related products

Product name	Package	Code No.
Uracil-DNA Glycosylase (UNG), Heat-labile <Glycerol Free>	200 U × 1	UNG-201
dUTP (100 mM)	0.5 mL × 1	UTP-101
dNTPs Mixture (A, C, G, U each 2 mM)	1 mL × 1	NTP-501
THUNDERBIRD™ Probe One-step qRT-PCR Kit	100 reactions	QRZ-101

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