Product Information	
Thermolabile UNG	
Part Number	G5030L
Concentration	1000 U/mL
Unit Size	250 units
Storage Temperature	-25ºC to -15ºC
Lot Number	(Shipment Specific)
Reference Number	(Shipment Specific)

Product Description:
Uracil-DNA Glycosylase (UNG) removes uracil from DNA and creates an abasic site in the DNA. Addition of UNG in PCR, qPCR, and RT-PCR can prevent carryover contamination which leads to false positive results. Customers performing high volume repetitive assays can benefit from addition of UNG to their workflow. It is compatible with most PCR and RT-PCR reaction buffers, and active in a broad buffer range.

Product Specifications				
G5030				
Assay	**SDS Purity**	**SS Exonuclease**	**DS Exonuclease**	**E. coli DNA Contamination**
Units Tested	**N/A**	**16**	**16**	**8**
Specification	>95%	<1%	<1%	<10 copies

Source of Protein: An E.coli strain carrying a cloned thermolabile uracil -N- glycosylase gene

Unit Definition: 1 unit is defined as the amount of Thermolabile UNG 2.0 required to release 1nmol of uracil from dU-containing DNA in one hour at 37°C

Molecular weight: 51,727 Daltons

Quality Control Analysis:
Unit Activity is measured using a 2-fold serial dilution method. Dilutions of enzyme were made in 1X reaction buffer (70mM Tris-HCl, 10mM NaCl, 1mM EDTA, 100 µg/mL BSA, pH 8.0 @ 25°C) and added to reactions containing a 3H-dUTP labeled 1.1kb PCR product in 1X reaction buffer. Reactions were incubated for 60 minutes at 37°C, plunged on ice, and analyzed using a TCA-precipitation method.

Protein Concentration (OD_{280}) is determined by OD_{280} absorbance.

Physical Purity is evaluated by SDS-PAGE of concentrated and diluted enzyme solutions followed by silver stain detection. Purity is assessed by comparing the aggregate mass of contaminant bands in the concentrated sample to the mass of the protein of interest band in the diluted sample.

Single-Stranded Exonuclease is determined in a 50 µL reaction containing a radiolabeled single-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.
Double-Stranded Exonuclease is determined in a 50 µl reaction containing a radiolabeled double-stranded DNA substrate and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Double-Stranded Endonuclease is determined in a 50 µL reaction containing 0.5 µg of plasmid DNA and 10 µL of enzyme solution incubated for 4 hours at 37°C.

Limitations of Use
This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. SDS sheets relevant to this product are available upon request.

E.coli 16S rDNA Contamination is evaluated using 5 µL replicate samples of enzyme solution denatured and screened in a TaqMan qPCR assay for the presence of contaminating *E.coli* genomic DNA using oligonucleotide primers corresponding to the 16S rRNA locus.

Supplied in 50 mM Tris-HCl, 50 mM NaCl, 1 mM DTT, 0.1 mM EDTA, 50% glycerol, pH 7.5 @ 25°C

Notes:

Treatment of reaction with UNG for 2 minutes at 25°C at the beginning of thermal cycling removes uracil residues from dU-containing DNA preventing it from serving as a template. Thermolabile UNG can be quickly heat-inactivated during reverse transcription at temperatures of at least 50°C for 10 minutes.

Usage instructions:

1. Follow standard PCR assay guidelines
   - Pipette gently
   - Mix thoroughly
   - Centrifuge the plate quickly before loading on instrument

2. Set up your quantitative PCR reaction per existing protocol. Thermolabile UNG (G5030-1000) is compatible with most PCR and RT-PCR reaction buffers.

3. Add 1µL Thermolabile UNG (G5030-1000) for each 50µL of master mix and vortex thoroughly.

Table below is an example of setting up a one step RT-qPCR reaction using a RNase H minus Reverse Transcriptase (such as Enzscript P7600L or StableScript P7720L) and an antibody based hot-start Taq DNA polymerase (such as our Phoenix Hot Start Taq P7590L)).

Step*	°C	Time	Cycles
UNG Treatment	25	2 min	1
Reverse transcription	50	10 min	1
RT inactivation and Initial Denaturation	94	3 min	1
Denaturation	94	15 sec	40
Annealing/Extension	60	60 sec	

**Optimal temperature and time for reverse transcription, RT inactivation and Taq activation, as well as Annealing/Extension condition need to be determined by end user.*

Related Products:

StableScript™ P7720L
StableScript One Step RT-qPCR P7730L
EnzScript P7600L
Phoenix Hot Start Taq DNA Polymerase P7590L

Limitations of Use
This product was developed, manufactured, and sold for *in vitro* use only. The product is not suitable for administration to humans or animals. SDS sheets relevant to this product are available upon request.