SOLIS BIODYNE

Salini UNG™ Uracil-N-Glycosylase

(1 U/µl)

Catalogue Number	Pack Size	Volume
31-01-0000S	25 U	25 µl
31-01-00100	100 U	100 µl
31-01-00100-5	5 x 100 U	5 x 100 µl

Store at –20°C upon receipt

Shipping:

At room temperature

Batch Number and Expiry Date:

See vial

Storage and Stability*:

- Routine storage at –20°C (–28°C to –18°C) until Expiry Date
- Stable at 4°C (2°C to 8°C) for 3 months
- Stable at room temperature (25°C) for 3 months
- Freeze-thaw stability: 30 cycles

Reaction setup:

At room temperature

Manufactured by Solis BioDyne, in compliance with the ISO 9001 and ISO 13485 certified Quality Management System.

Product description:

- Salini UNG™ Uracil-N-Glycosylase is a heat-labile enzyme that eliminates uracil from dUTP-DNA by pinch-push-pull strategy, catalyzing the hydrolysis of the N-glycosylic bond and creating an abasic site.
- Salini UNG™ Uracil-N-Glycosylase is an exceptionally stable enzyme due to a unique patented genetic modification in the polypeptide structure called the Stability TAG**. The enzyme is stable for at least 2 weeks at 37°C and 3 months at 25°C.
- Protein sequence of Salini UNG™ Uracil-N-Glycosylase originates from the bacteria genus *Salinivibrio* which is frequently found in hypersaline environments.
- Salini UNG™ Uracil-N-Glycosylase is a heat-labile enzyme inactivated by heat treatment at 70°C for 5 min. No reactivation of the enzyme is detected if stored at 4°C or 25°C for 48h or at -20°C for at least 1 month.
- Salini UNG™ Uracil-N-Glycosylase is compatible with Sanger sequencing. Storage at 4°C for at least 24h and at -20°C for 1 month does not show degradation of the amplification product. However, we recommend purification of PCR product prior to sequencing.
- Salini UNG™ Uracil-N-Glycosylase does not compromise the performance of a range of DNA polymerases and reverse transcriptases. The enzyme removes uracil (U) incorporated into a single- or double-stranded DNA. Degraded dUTP-DNA cannot be used as a template for further amplification. A combination of these properties is widely used as a part of the

carryover contamination prevention strategy. Salini UNG™ Uracil-N-Glycosylase can be combined with (q)PCR and RT-(q)PCR mixes with dUTP.

- Salini UNG™ Uracil-N-Glycosylase is an extremely fast enzyme that catalyzes the degradation of 5 µg of uracil-containing dsDNA by 50% in 15 seconds at 37°C.

Applications:

- Widely used to eliminate carryover contamination in PCR and LAMP
- Enhancer of cloning efficiency of PCR products
- Site-directed mutagenesis
- As a probe for protein-DNA interaction studies
- Glycosylase-mediated single nucleotide polymorphism detection (GMPD)
- Study of DNA repair and mutation detection
- SNP genotyping

Recommentations for use:

- **Working temperature.** The optimal working temperature of Salini UNG™ Uracil-N-Glycosylase is strongly dependent on the reaction mixture composition and pH. For (q)PCR applications we recommend keeping the reaction temperature within 25–40°C.
- **Working concentration.** For qPCR application, we recommend using enzyme at a final concentration of 0.025–0.04 U/µl, for endpoint PCR at a final concentration of 0.005–0.01 U/µl. LAMP reactions result in 100x more product compared to PCR, therefore for LAMP we recommend scaling up. For other applications, we recommend adjusting protocol according to the unit definition, where one unit is defined as amount of Salini UNG™ Uracil-N-Glycosylase that catalyzes the degradation of 5 µg uracil-containing dsDNA by 50% in 15 seconds at 37°C.
- **UNG treatment protocol.** If the reaction setup is at room temperature, an additional treatment step is not required. For most qPCR protocols 30 seconds at 25°C is sufficient for UNG treatment prior qPCR cycling.
- **Enzyme inactivation**. The enzyme is inactivated at 70°C for 5 min. Inactivation at higher temperatures is not recommended.
- **Downstream analysis of amplification product.** Amplification products should be stored at –20°C to avoid degradation. Storage at room temperature for 24 hours has no detrimental effect on the amplification products.

Considerations for carryover contamination prevention

The most important source of carryover contamination is from the repeated amplification of the same target sequence. PCR and LAMP are sensitive and specific nucleic acid amplification methods. PCR typically produces ~0.2 mg, and LAMP results in ~ 10–20 mg of product.

1. Separate working areas: pre-PCR (aliquoting and mixing), amplification area, post-PCR (analysis). Move in one direction starting from the pre-PCR area.
2. Briefly centrifuge tubes before opening, it helps to reduce the contamination from aerosols. Proper pipetting technique helps to avoid splashing and contamination of the pipette.
3. Use master mixes to minimize pipetting steps and plastic waste.
4. Include a proactive contamination prevention method. To prevent carryover contamination, dUTPs must be incorporated into the previous amplification product. For best results, substitute 100% of dTTPs with dUTPs. However, not all DNA polymerases can efficiently incorporate dUTPs instead of dTTPs. Solis BioDyne's SolisFAST® DNA Polymerase, included in SolisFAST® Master Mixes, efficiently incorporates 100% dUTPs without compromising the polymerization speed. SolisFAST® PCR and qPCR Master Mixes with UNG already contain 100% dUTPs as well as Salini UNG™ Uracil-N-Glycosylase and enable highly efficient and fast UNG treatment and PCR.

Step-by-step guidelines PCR protocol with Salini UNG™:

1. Thaw reagents, template DNA, primers, and nuclease-free water. Mix each component by gentle vortexing or pipetting up and down, then centrifuge briefly.
2. Prepare a reaction mix. Add all required components.

Component	Final concentration in reaction	
	PCR	qPCR
PCR Master Mix[1]	1x	1x
dUTP (or dUTP/dTTP mix)[2], dATP, dCTP, dGTP	200 µM of each	200 µM of each
Forward/reverse primer	100–300 nM of each	80–200 nM of each
Salini UNG™ Uracil-N-Glycosylase	0.005–0.01 U/ µl	0.025–0.04 U/ µl
Template DNA[3]	Variable	Variable
Nuclease-free water	up to 20 µl	up to 20 µl
Total reaction volume	**20 µl**	**20 µl**

[1] DNA polymerase must be able to incorporate dUTP into amplification product.

[2] For best results substitute 100% of dTTPs with dUTPs. Depending on the DNA polymerase 20%, 40%, 60% and 80% of dUTPs relative to dTTPs can be tested.

[3] Concentration of cDNA 0.1 pg/µl–10 ng/µl; gDNA 10 pg/µl–4 ng/µl

3. Mix the reaction mix thoroughly, then centrifuge briefly.
4. Place plates or tubes with reaction mix to thermal cycler and run respective protocol.

   4.1. Optional step. If you set-up reaction on ice, add 30 seconds at 25°C for UNG treatment prior amplification. Skip this step if you set-up reaction at room temperature.

Source:

E. coli strain that carries a Salini UNG™ Uracil-N-Glycosylase gene overproducing plasmid.

Unit definition:

One unit is defined as the amount of Salini UNG™ Uracil-N-Glycosylase that catalyzes the degradation of 5 µg uracil-containing dsDNA in 15 seconds at 37°C by 50%.

Safety precautions:

Please refer to Safety Data Sheet for more information.

Technical support:

Contact your sales representative for any questions or send an email to support@solisbiodyne.com

For research use only. Not for use in diagnostic procedures.

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***Product stability** is assessed using routine QC assays and QC criteria set forth in the product specification and are intended to provide guidelines for shipping and storage conditions only. Customer or its designee shall be responsible for conducting all necessary stability testing applicable to their assay and/or QC criteria, and to comply with any applicable regulatory requirements or guidelines. Such stability testing shall include testing to validate the lead times for shipment, the shelf life of, and the product specifications applicable to shipment, storage and handling of the assay assembled and packed by the customer.

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**Covered by patent EP2501716, made following the methods of US Patent No 9,321,999.

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