

Version 1 Last updated 26 August 2016

# ab215390 Recombinant E. coli UNG protein kit

1 Unit of the enzyme catalyzes the release of 1 nanomole of uracil-containing DNA template in 60 min at 37°C.

This product is for research use only and is not intended for diagnostic use.

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## 1. Overview

E. coli UNG protein kit (ab215390) catalyzes the release of free Uracil from Uracil-containing DNA. UNG efficiently hydrolyzes uracil from single-stranded or double-stranded DNA, but not from oligomers (6 fewer bases). It is active over a broad pH range with an optimum at pH-8.0, doesn't require divalent cation, and is inhibited by high ionic strength (>200 mM). The abasic sites formed in DNA by UNG may be cleaved by heat, alkali-treatment or endonucleases that cleave specifically at abasic sites. This product can be used for: Glycosylase mediated single nucleotide polymorphism detection (GMPD). Site-directed mutagenesis. As a probe for protein-DNA interaction studies. Rapid and efficient cloning of PCR products. Elimination carry-over contamination in PCR. Inactivated by heating at 95°C for 10min. Enzyme activity is partially restored at temperatures lower than 55°C.

1 Unit is defined as the amount of UDG enzyme that catalyzes release of 60 pmol of Uracil per minute from double-stranded, uracil-containing DNA in a total reaction volume of 50  $\mu$ L in 30 minutes at 37°C in 1X UNG Reaction Buffer (200 mM Tris-HCl (pH 8.0 at 25°C), 10 mM DTT and 10 mM EDTA) with 1 Unit of UNG and 0.2  $\mu$ g [3H]-Uracil DNA (104- 105 cpm/ $\mu$ g).

The specific activity is 5 U/ $\mu$ L.

The UNG protein was purified by standard chromatographic techniques.

## 2. Precautions

**Please read these instructions carefully prior to beginning the assay.**

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

## 3. Storage and Stability

**Store kit at -20°C immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.**

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

## 4. Limitations

- Kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.
- UNG does not hydrolyze uracil from oligomers (6 fewer bases).

## 5. Materials Supplied

Item	Quantity	Storage Condition (Before prep)	Storage Condition (After prep)
10X UNG Reaction Buffer	2 mL	-20°C	-20°C
UNG Enzyme	1 Vial	-20°C	-20°C
UNG Storage Buffer	2 mL	-20°C	-20°C

## 6. Technical Hints

- Avoid foaming or bubbles when mixing components.
- Avoid cross contamination of samples or reagents by changing tips between samples and reagents.
- All samples should be mixed thoroughly and gently.
- Avoid multiple freeze/thaw of reagents and samples.
- This kit is sold based on number of International units of UNG Enzyme. Review the booklet completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.

## 7. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.
- Prepare only as much reagent as is needed on the day of the experiment.

### 7.1 10X UNG Reaction Buffer

Prepare 1X UNG Reaction buffer. 2 mL, store at -20°C.

### 7.2 UNG Enzyme

Ready to use. 1 Vial. store at -20°C.

### 7.3 UNG Storage Buffer

Ready to use. 2 mL. store at -20°C.

## 8. Notes

## Technical Support

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