

Cod Uracil-DNA Glycosylase (Cod UNG)

Product Data

Source

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

Concentration

50 U/μl ±5 U

Specific activity

> 500 000 U/mg

Unit definition

One unit of Cod UNG is defined as the amount of enzyme required to release 1 nmol uracil from uracil-containing DNA per hour at 37°C using an assay buffer containing 70 mM Tris-HCl, pH 7.5 (at 25°C), 10 mM NaCl, 1 mM EDTA, 100 μg/ml BSA.

Reaction buffer

Cod UNG is not dependent of any cofactors or divalent cations for activity and works in common PCR and RT-PCR buffers.

Storage

Minimum shelf life is 2 years at -20°C. In practice we find that storage at 4°C is possible for at least 6 months. The enzyme activity also tolerates multiple freeze-thaw cycles.

Storage buffer

20 mM Tris-HCl, pH 7.5 (at 25°C)

50 mM NaCl

1 mM DTT

0.1% (v/v) Triton X-100

50% (v/v) glycerol

Purity

The enzyme is purified to apparent homogeneity by SDS-PAGE, and has been tested free for contaminating endo- and exonucleases.

Quality control

Endonuclease

50 U of Cod UNG is incubated with 1 μg pGEM plasmid in 40 mM Tris-HCl, pH 8.0, 20 mM MgCl₂ and with or without

5 mM DTT for 2 hours at 37°C in a total reaction volume of 50 μl. Endonuclease is not detected when there is no visual difference in banding pattern by agarose gel analysis compared to control.

Exonuclease

50 U of Cod UNG is incubated with denatured ³H-labelled DNA in 50 mM Tris-HCl, pH 7.5, 10 mM MgCl₂, 1 mM DTT for 2 hours at 37°C in a total reaction volume of 20 μl. Exonuclease is not detected if released radioactivity is less than background plus 2 x SD.

Properties

Cod UNG hydrolyses the N-glycosylic bond between the deoxyribose sugar and the base in uracil-containing DNA leaving an apyrimidinic site in DNA. Cod UNG hydrolyses uracil from both single- and double stranded dU-containing DNA, but not from RNA.

Heat inactivation

The enzyme is completely and irreversibly heat inactivated by incubating for 10 min at 50°C.

Usage

Suitable amount of enzyme is application dependent, but is normally in the range between 0.1-1 U/50 μl reaction.

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