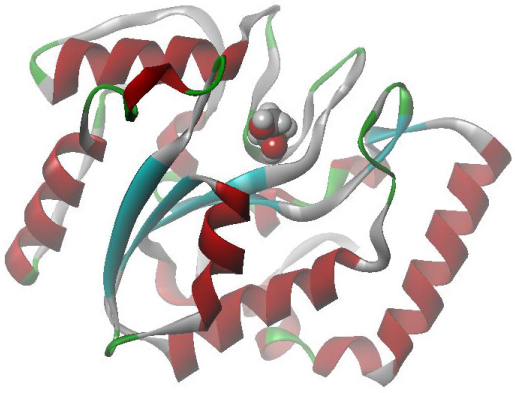
Cod Uracil-DNA Glycosylase (Cod UNG)

- Heat-labile
- Completely and irreversibly inactivated
- Highly advantageous for use in qRT-PCR and qPCR
- Does not degrade PCR products post-PCR
- Tested free of contaminating nucleases

"The only UNG that is fully inactivated by heat"

Recombinant Atlantic Cod Uracil-DNA Glycosylase (Cod UNG) is a heat-labile UNG enzyme for use in molecular biology research and diagnostics. The enzyme is produced in an *E. coli* (ung$^-$) strain expressing a recombinant gene encoding Cod UNG. The key properties of this enzyme are the rapid, complete and irreversible inactivation upon heat treatment and the high activity at low temperatures. Unlike all other available UNG enzymes, Cod UNG does not reactivate after heat inactivation.



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 is not active on uracil in RNA.

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.
Activity	Cod UNG is highly active in the temperature range from 20 – 40°C. Optimal activity is at pH 7.5 and 50 mM NaCl.
Heat inactivation	Complete and irreversible inactivation of Cod UNG is accomplished by incubating the enzyme at 50°C for 10 min.
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.
Specific activity	> 500 000 U/mg
Molecular weight	28 kDa
Purity	Cod UNG is tested free of contaminating nucleases.

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Use of Cod UNG in qRT-PCR

A prerequisite for using Cod UNG for contamination control in qRT-PCR is that the enzyme is sufficiently heat-labile to inactivate quickly at the RT-temperature. The Cod UNG is advantageous to use in both qPCR and qRT-PCR assays and is able to remove more than 10^8 copies of contaminating DNA without affecting the sensitivity (C_t) of the assay. The fast heat inactivation and the high activity at low temperature make it possible to do the Cod UNG incubation step at room temperature for only 5 minutes prior to the amplification reaction.

PCR product stability after Cod UNG treatment

The complete and irreversible heat-inactivation makes Cod UNG ideal for any qPCR or qRT-PCR application where the products need to remain intact for further analysis such as cloning, sequencing or genotyping using single-nucleotide primer extension technology. It is also highly advantageous when the samples have to be stored for prolonged time post-amplification.

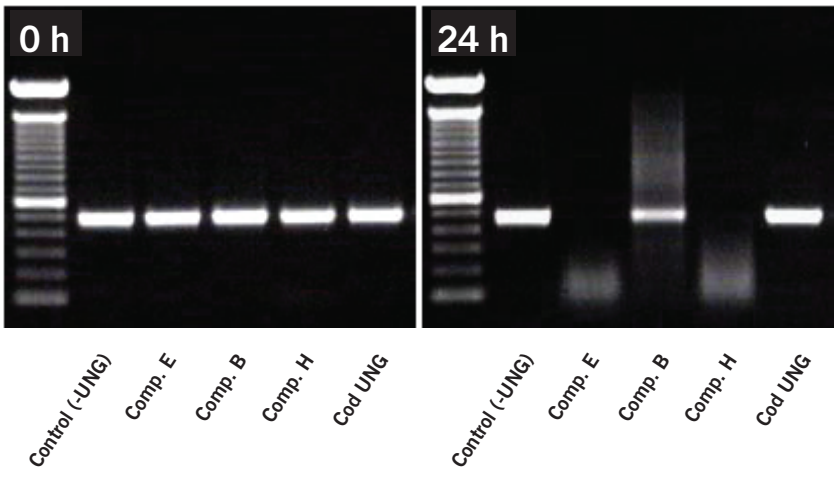

Figure 2 Integrity analysis of dU-containing PCR-products. PCR reactions were added 0.5 U Cod UNG and PCR products were stored at room temperature (RT) for 0 hour and 24 hours post-PCR and analyzed by agarose gel electrophoresis. Cod UNG is the only enzyme that leaves the PCR product intact post-PCR.

No effect on sensitivity

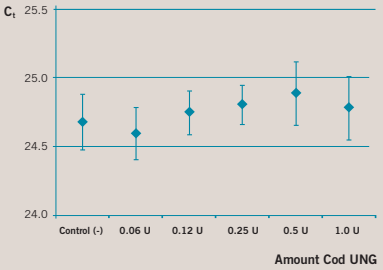

Figure 1 qRT-PCR using 1 ng total RNA as a template. Cod UNG preincubation step was 5 minutes at 25°C. RT-step was performed at 50°C, and total reaction volume was 25 µl. Adding Cod UNG give no significant increase in C_t.

Adding 0.12 U Cod UNG removes 10^8 copies of contaminating DNA after a 5 min at 25°C preincubation step.

References

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