Cod Uracil-DNA Glycosylase (Cod UNG)

- The only UNG that is completely and irreversibly heat inactivated
- Heat-labile without any addition of agents or inhibitors
- Eliminates carry-over contamination in both PCR and RT-PCR
- Enables downstream post-PCR analysis such as cloning and sequencing.

"The only UNG useful in RT-PCR"

Properties

Cod UNG hydrolyses the N-glycosylic bond between the deoxyribose sugar and the base in uracilcontaining DNA leaving an abasic (apyrimidinic) site in DNA. Cod UNG is not active on uracil in RNA.

The high activity combined with a fast irreversibly heat-inactivation step makes the enzyme ideal for contamination control in PCR- and RT-PCR reactions.

Unlike all other available UNG enzymes, Cod UNG does not reactivate after heat-inactivation. This enables contamination control in amplification reactions also where subsequent post-PCR analysis is required.

Source: Arctic cod origin, recombinantly produced in E. coli.

Activity: Cod UNG is highly active in the temperature range from 20-40°C. No cofactors or divalent cations are required for activity. The enzyme is active in most PCR- and RT-PCR buffers. The pH working range is between 6.5-9.0. Optimal activity is at pH 7.5 and 50 mM NaCl.

Heat inactivation: Cod UNG is completely and irreversibly inactivated by incubating the enzyme at 55°C for 20 min.

Storage: Minimum shelf life is 2 years at -20°C. Storage at 4°C is possible for at least 6 months. The enzyme also tolerates multiple freeze-thaw cycles.

Purity: Cod UNG highly pure and is tested free of contaminating nucleases. **Specific activity:** > 500 000 Units/mg.

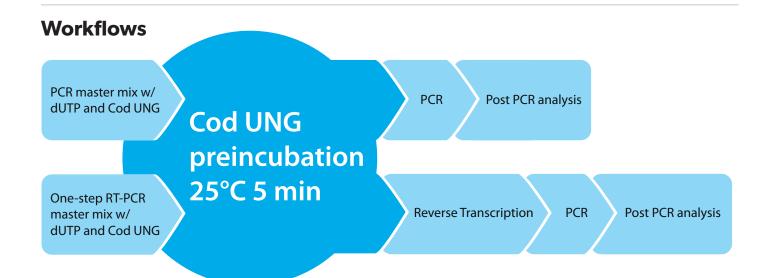
Unit definition: One unit of UNG is defined as the amount of enzyme required to release 1 nmol uracil from uracil-containing DNA per hour at 37°C.



PO Box 662 South Yarra VIC 3141 Australia **WWW.SCientifix.com.au**

Free call: 1800 007 900 T: +61(0)3 8540 5900 F: +61(0)3 9543 7827 E: info@scientifix.com.au





Use of Cod UNG in RT-PCR

A prerequisite for using UNG in RT-PCR contamination control, is that the enzyme is sufficiently heat-labile to quickly inactivate at the temperatures used for reverse transcription. The easily heat-inactivated Cod UNG makes it possible to use contamination control also in RT-PCR, being able to remove more than 10⁸ copies of contaminating DNA without affecting the sensitivity (Cq) of the assay. Applying Cod UNG in a RT-PCR is done by a 5 min Cod UNG preincubation step at room temperature prior to the amplification reaction.

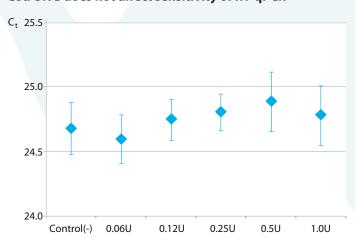




Figure 1: qRT-PCR using 1 ng total RNA as a template. Cod UNG preincubation step was 5 minutes at 25°C. RT-step was performed at 50°C.

PCR product stability after Cod UNG treatment

The complete and irreversible heat-inactivation of Cod UNG enables the combination of contamination control and an array of post-PCR applications such as cloning, sequencing or genotyping. As Cod UNG is the only commercially available UNG that does not reactivate postamplification, PCR-products remain intact and can be stored for prolonged period of time before further analysis.

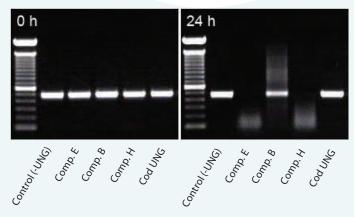


Figure 2: Integrity analysis of dU-containing PCR-products. PCR reactions were added 0.5 U Cod UNG and PCR products were stored at room temperature (RT) for 0 hour and 24 hours post-PCR and analyzed by agarose gel electrophoresis. Cod UNG is the only enzyme that leaves the PCR product intact post-PCR.

For more information visit: www.arcticzymes.com/codung

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