

Recombinant Thermostable dUTPase Pyrococcus Fruriosus

Catalog No.	CSI12676	Quantity:	500 U
	CSI12677		1000 U
	CSI12678		2000 U

Alternate Names: Thermostable dUTPase, dUTPase.

Description: Thermostable dUTPase (pyrococcus fruriosus) maximizes the efficiency of high-fidelity PCR (using proofreading DNA polymerases). It removes contaminating dUTP present in PCR reactions and dNTP solutions. The presence of dUTPase in a proofreading DNA polymerase reaction can prevent dUTP misincorporation by maintaining dUTP levels below their inhibitory concentrations despite the constant generation of the molecule by the spontaneous deamination of dCTP. The incorporation of dUTP into PCR products causes mutations within the amplified product, proofreading polymerases to stall and slows down non-proofreading polymerases such as Taq. The dUTPase increase in PCR product yield, length and fidelity enables further down-stream applications. These effects make dUTPase useful in PCR fidelity and yield-sensitive applications such as cloning and subsequent recombinant protein technology, and gene expression analysis (semi-quantitative RT-PCR techniques and real-time PCR analysis), where small differences in product accumulation can have a significant impact on gene expression analysis. dUTPase is specific for dUTP and is critical for the fidelity of DNA replication and repair. dUTPase hydrolyzes dUTP to dUMP and pyrophosphate, simultaneously reducing dUTP levels and providing the dUMP for dTTP biosynthesis.

Physical Appearance: Sterile filtered liquid formulation.

Source: *E. coli*

Formulation: dUTPase is supplied in 20 mM Tris-HCl (pH 8.2) + 1 mM DTT + 0.1 mM EDTA + 100 mM KCl + 0.1% Nonidet P40 + 0.1% Tween 20 and 50% glycerol at concentration of 10 u/ul of the enzyme.

Purity: Greater than 97.0% as determined by
(a) Analysis by RP-HPLC.
(b) Analysis by SDS-PAGE.

Specific Activity: 103 u/ug.

Activity: (A) Measured by its ability to hydrolyze dUTP to dUMP in reaction buffer 20 mM Hepes pH 7.5 + 150 mM KCl + 5 mM MgCl₂ + 10 mM dUTP at 85 Centigrade for 1 hour. The PPi production was quantified by using the enzymatic determination kit from Sigma.
(B) Enhancing PCR amplification: 50 ul of Pfu PCR reaction system with 1-3 u of dUTPase (<3.0 kb) or 4-6 u dUTPase (>3.0 kb) to amplify genomic DNA target up to 15-19 kb in length. (High concentrations of dUTPase will inhibit PCR reaction!)

Unit Definition: One unit of enzyme catalyzes hadrylization of 10 nanomoles of dUTP to dUMP in one hour at 85 Centigrade.

Storage & Stability: Two years when stored at -20°C, 2 weeks at 4°C

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