

dNTP Set

Store at -20°C

Cat. No.

PGN018

Pack Size

4 x 25 µmol

Volume:

0.25 mL of 100 mM dATP

0.25 mL of 100 mM dCTP

0.25 mL of 100 mM dGTP

0.25 mL of 100 mM dTTP

Description

The set consists of 100 mM aqueous solutions of dATP, dCTP, dGTP and dTTP each supplied in a separate vial. The nucleotide solutions are titrated to pH 7.3-7.5 with NaOH. Since the nucleotides are provided separately, the dNTP Set offers maximum flexibility in preparation of reaction mixes for different applications.

Applications

For use in PCR, real-time PCR, high fidelity and long PCR, LAMP-PCR, cDNA synthesis, RT-PCR, RDA, MDA, DNA labeling, and DNA sequencing.

General Characteristics

- dATP** C10H13N5O12P3Na3; MW = 557.2;
λ_{max}=259 nm; ε=15.4×10³ M⁻¹cm⁻¹ at pH 7.0;
- dCTP** C9H13N3O13P3Na3; MW = 533.1;
λ_{max}=271 nm; ε=9.1×10³ M⁻¹cm⁻¹ at pH 7.0.
- dGTP** C10H13N5O13P3Na3; MW = 573.2;
λ_{max}=253 nm; ε=13.7×10³ M⁻¹cm⁻¹ at pH 7.0.
- dTTP** C10H14N2O14P3Na3; MW = 548.1;
λ_{max}=267 nm; ε=9.6×10³ M⁻¹cm⁻¹ at pH 7.0.

Important Note

Mix well each dNTP solution prior to use.

Preparation of different concentration dNTP mixtures

dNTP mixture to be prepared	Volumes of dNTP Set, µL				Water, nuclease-free, µL	Total volume of dNTP mixture, µL
	100 mM dATP	100 mM dGTP	100 mM dCTP	100 mM dTTP		
2 mM of each dNTP	10	10	10	10	460	500
	100	100	100	100	4600	5000
	250	250	250	250	11500	12500
10 mM of each dNTP	10	10	10	10	60	100
	100	100	100	100	600	1000
	250	250	250	250	1500	2500
25 mM of each dNTP	10	10	10	10	–	40
	100	100	100	100	–	400
	250	250	250	250	–	1000

Getting 0.2 mM dNTP in PCR

Volume of PCR Mixture	dNTP Mixture to be added to PCR		
	2 mM	10 mM	25 mM
25 µL	2.5 µL	0.5 µL	0.2 µL
50 µL	5 µL	1 µL	0.4 µL
100 µL	10 µL	2 µL	0.8 µL

CERTIFICATE OF ANALYSIS

Purity is ≥99% for each dNTP, determined by HPLC.

Concentration is 100±3 mM for each dNTP, determined spectrophotometrically.

pH is 7.3-7.5 for each dNTP, determined according to Ph. Eur. 2.2.3

Endo- and exonucleases. Each dNTP was tested by incubation of single stranded and double stranded radiolabeled oligonucleotides with 1ul of 20 mM dNTP for 4 hours at 37°C and separation of reaction mixtures on a denaturing polyacrylamide gel. Phosphorimaging has not detected DNA degradation.

Ribonucleases. Each dNTP was tested by incubation of 2,000 bases RNA transcript with 1ul of 20 mM dNTP at 37°C for 4 hours and separation of reaction products on an agarose gel. There was no decrease in RNA transcript band intensity compared to control.

Nicking activities. Each dNTP was tested by incubation of 1 µg of supercoiled pUC19 DNA with 1ul of 20 mM dNTP at 37°C for 17 hours and separation of reaction mixtures on an agarose gel. Neither linearised plasmid, nor relaxation of supercoiled plasmid was detected as compared to control.

E.coli DNA. Quantitative PCR test on ABI Prism 7000 SDS, which uses amplification of *E.coli* 23S rRNA gene fragment did not detect *E.coli* DNA.

Human DNA. Quantitative PCR test on ABI Prism 7000 SDS, which uses amplification of human genomic DNA fragment did not detect human DNA.

Functional test.

1. PCR amplification of a single-copy gene fragment (1 kb) from 10 copies of human genomic DNA using *Pfu* DNA polymerase.
2. PCR amplification of 5 kb DNA fragment from series of lambda DNA dilutions using *Pfu* DNA polymerase.

