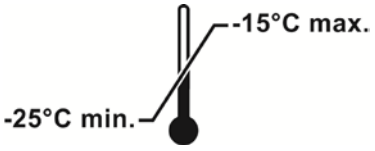
2.5 mM dNTP Mix, PCR Grade

Lucigen®
Simplifying Genomics

Technical Specifications

Catalog No.: 30030-0 50 µl Trial Size
30030-1 2 ml (2 X 1 ml)
30030-2: 10 ml (5 X Cat. No. 30030-1)
30030-3: 20 ml (10 X Cat. No. 30030-1)



Store at –20ºC.
For *In Vitro* Research Use Only.
Not for Drug or Diagnostic use. Not for use in humans or animals.

Product Description	2.5 mM dNTP Mix, PCR Grade, is a solution containing the sodium salts of dATP, dCTP, dGTP and dTTP, each at a concentration of 2.5 mM in water: the total concentration of nucleotides being 10 mM (pH 8.3).
Storage and usage conditions	Store the product at -20^{O}C. Avoid frequent freeze and thaw cycles. Mix well prior to use.
Stability	2.5 mM dNTP Mix, PCR Grade, is stable for one year from the date received if stored at -20ºC.
Recommended Reaction Conditions	200 µM each dNTP; 1X Reaction Buffer; 1 µM primers and 1 - 2.5 U Thermostable DNA Polymerase.
Applications	PCR, RT-PCR, Reverse Transcription, DNA labeling reactions and Sequencing/Cycle Sequencing.
Absence of Endonuclease or Nicking Activity	Incubation of 20 µl of 2.5 mM dNTP Mix, PCR Grade, with 1 µg of supercoiled pBR322 DNA for 16 hours at 37ºC resulted in no detectable conversion to relaxed or linear forms by agarose gel electrophoresis.
Absence of Exonuclease Activity	Incubation of 20 µl of 2.5 mM dNTP Mix, PCR Grade, with 1 µg of HindIII-cut lambda DNA for 16 hours at 37ºC resulted in no smearing of bands on agarose gels.
Absence of Ribonuclease Activity	Incubation of 20 µl of 2.5 mM dNTP Mix, PCR Grade, with fluorescent labeled RNA substrate resulted in no detectable RNase activity.
Quality Control	The 2.5 mM dNTP Mix, PCR Grade, is tested in DNA amplification using a variety of templates and primers.
Purity	>99% pure.

PLEASE NOTE
Some applications in which Lucigen's 2.5 mM dNTP Mix, PCR Grade, can be used may be covered by patents issued and applicable in the United States and other countries. Because purchase of this product does not include a license to perform any patented application, users of this product may be required to obtain a patent license depending upon the particular application in which the product is used. The PCR process is the subject of European Patent Nos. 201,184 and 200,262 owned by Hoffman-LaRoche. Those patents expired on March 28, 2006. The corresponding PCR process patents in the United States expired on March 29, 2005. It is the sole responsibility of the buyer to ensure that use of the product does not infringe the patent rights of third parties.

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Warranty

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PCR Setup Protocol

1) PCR amplification is performed by adding Template DNA (10-50 ng of plasmid DNA; 50-200 ng of genomic DNA), reaction buffer, Forward Primer (100 pmol/µl) and Reverse Primer (100 pmol/µl), dNTPs**,** thermostable DNA polymerase and water.

2) Reaction Setup. Set up PCR amplifications of the desired size (on ice for best results), according to the following chart:

	25 µl Reaction	50 µl Reaction	100 µl Reaction	Final concentration
DNA template (10 ng/µl)	1.0 µl	1.0 µl	1.0 µl	< 50 ng
10 X Reaction Buffer containing 15 mM $MgCl_2$	2.5 µl	5.0 µl	10.0 µl	1 X
Forward Primer (100 pmol/µl)	0.25 µl	0.5 µl	1.0 µl	1 pmol/ µl
Reverse Primer (100 pmol/µl)	0.25 µl	0.5 µl	1.0 µl	1 pmol/ µl
2.5 mM dNTP Mix, PCR Grade	2.0 µl	4.0 µl	8.0 µl	0.2 mM each
Thermostable DNA Polymerase (5U/ µl)	0.5 µl	0.5 µl	0.5 µl	2.5U
Water, Nuclease-free	18.5 µl	38.5 µl	78.5 µl	---

3) Gently mix the PCR components in a thin-walled reaction tube and spin briefly in a microcentrifuge. Add a drop of mineral oil if the thermocycler does not have a heated lid.

4) PCR Cycling Conditions

Pre-heat the thermocycler to 94°C.

Cycling step	Temperature	Time	# of Cycles
Initial Denaturation	94°C	2 min	1
Denaturation	94°C	15-30 sec	25 – 35
Annealing*	50-65°C	15-30 sec	
Extension	72°C	1 min/kb	
Final Extension	72°C	5 -10 min	1
Hold	4°C	Indefinitely	1

***Anneal at T_m of primer ± 2°C.**

5) After completion of the PCR, a 5-µl aliquot of the reaction is loaded onto an agarose gel for analysis or size selection.