Manual

10 mM dNTP Set, PCR Grade

For Research Use Only. Not for use in diagnostic procedures.

IMPORTANT
-20 ºC storage required
immediately upon receipt

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

Manual

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Contents

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1. Product description

10 mM dNTP Set, PCR Grade, contains separate vials of 10 mM sodium salt solutions of each of dATP, dCTP, dGTP and dTTP (pH 8.3).

2. Product specifications

Stability: 10 mM dNTP Set, PCR Grade, is stable for one year from the date received if stored at -20 °C.

Recommended reaction conditions: 200 µM each dNTP; 1X reaction buffer; 1 µM primers and 1 - 2.5 U thermostable DNA polymerase.

Absence of endonuclease or nicking activity: Incubation of 20 µL of each 10 mM dNTP, PCR Grade, with 1 µg of supercoiled pBR322 DNA for 16 hours at 37 °C resulted in no detectable conversion to relaxed or linear forms by agarose gel electrophoresis.

Absence of exonuclease activity: Incubation of 20 µL of each 10 mM dNTP, PCR Grade, with 1 µg of *Hin*dIII-cut lambda DNA for 16 hours at 37 °C resulted in no smearing of bands on agarose gels.

Absence of ribonuclease activity: Incubation of 20 µL of each 10 mM dNTP, PCR Grade, with fluorescent labeled RNA substrate resulted in no detectable RNase activity.

Quality control: The 10 mM dNTP Set, PCR Grade, is tested in DNA amplification using a variety of templates and primers.

Purity*:* >99% pure.

3. Product designations and kit components

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
10 mM dNTP Set, PCR Grade	750 µL of each dNTP	30029-1	10 mM dATP	F95648-5	750 µL
			10 mM dTTP	F95649-5	750 µL
			10 mM dGTP	F95650-5	750 µL
			10 mM dCTP	F95651-1	750 µL
	5 X Cat. No. 30029-1	30029-2	10 mM dATP	F95648-5	3.75 mL
			10 mM dTTP	F95649-5	3.75 mL
			10 mM dGTP	F95650-5	3.75 mL
			10 mM dCTP	F95651-1	3.75 mL

4. Storage conditions

Store all kits and components at -20 °C. Avoid frequent freeze and thaw cycles. Mix well prior to use.

5. Applications

1. PCR
2. RT-PCR
3. Reverse transcription
4. DNA labeling reactions
5. Sequencing/cycle sequencing

6. PCR setup protocol

6.1. 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.

6.2. **Reaction setup.** Set up PCR amplifications of the desired size (on ice for best results), according to the following:

				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 (pmol/µL)	0.25 µL	0.5 µL	1.0 µL	1 pmol/µL
Reverse primer (pmol/µL)	0.25 µL	0.5 µL	1.0 µL	1 pmol/µL
10 mM dNTP Set (add indicated amount for each dNTP)	0.5 µL	1.0 µL	2.0 µL	0.2 mM each
Thermostable DNA polymerase (5 units/µL)	0.5 µL	0.5 µL	0.5 µL	2.5 U
Water, nuclease-free	18.5 µL	38.5 µL	78.5 µL	—
Total reaction volume	25.0 µL	50.0 µL	100.0 µL	

6.3. 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 thermal cycler does not have a heated lid.

6.4 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	
Annealing*	50-65 °C	15-30 sec	25-35
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.

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

7. Technical support

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

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