

# Manual

## 10 mM dNTP Set, PCR Grade

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

**IMPORTANT**  
**-20 °C storage required**  
immediately upon receipt

# Manual

10 mM dNTP Set, PCR Grade

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# Manual

## 10 mM dNTP Set, PCR Grade

### 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 *HindIII*-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.

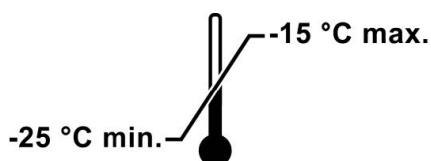
**Purity:** >99% pure.

### 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.



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## 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/ $\mu$ L) and reverse primer (100 pmol/ $\mu$ L), 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/ $\mu$ L)	1.0 $\mu$ L	1.0 $\mu$ L	1.0 $\mu$ L	<50 ng
10 X Reaction Buffer containing 15 mM MgCl <sub>2</sub>	2.5 $\mu$ L	5.0 $\mu$ L	10.0 $\mu$ L	1 X
Forward primer (pmol/ $\mu$ L)	0.25 $\mu$ L	0.5 $\mu$ L	1.0 $\mu$ L	1 pmol/ $\mu$ L
Reverse primer (pmol/ $\mu$ L)	0.25 $\mu$ L	0.5 $\mu$ L	1.0 $\mu$ L	1 pmol/ $\mu$ L
10 mM dNTP Set (add indicated amount for each dNTP)	0.5 $\mu$ L	1.0 $\mu$ L	2.0 $\mu$ L	0.2 mM each
Thermostable DNA polymerase (5 units/ $\mu$ L)	0.5 $\mu$ L	0.5 $\mu$ L	0.5 $\mu$ L	2.5 U
Water, nuclease-free	18.5 $\mu$ L	38.5 $\mu$ L	78.5 $\mu$ L	—
Total reaction volume	25.0 $\mu$ L	50.0 $\mu$ L	100.0 $\mu$ L	

- 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<sub>m</sub> of primer  $\pm$  2 °C.

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6.5. After completion of the PCR, a 5  $\mu$ L 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](mailto:techsupport@lgcgroup.com).

#### PLEASE NOTE

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