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Roche

7-Deaza-2´-deoxy-guanosine-5´-triphosphate

Lithiumsalt

Cat. No. 10 988 537 001 2 µmol (200 µl)

Version February 2007

Store at −15 to −25°C

1. What this Product Does

Contents

Lithium salt, 10 mM aqueous solution, pH 7

Storage and Stability

The 7-Deaza-2´-deoxy-GTP is stable at −15 to −25°C through the expiration date printed on the label. The 7-Deaza-2´-deoxy-GTP is shipped on dry ice.

Application

7-Deaza-dGTP is a substrate for most DNA polymerases, including Taq DNA polymerase. Incorporation of 7-deaza-dGTP into DNA alters the fluorescent staining and electrophoretic mobility (3) of the DNA.

7-Deaza-dGTP is used in the dideoxy-chain termination sequencing methods, in place of dGTP to overcome compression problems in gel electrophoresis when sequencing GC-rich stretches of DNA. Comparison of 7-deaza-dGTP with dGTP and dITP showed that 7-deaza-dGTP gives enhanced resolution compared with dGTP, and provides increased legibility over long sequence regions compared with dITP. For sequencing reactions, dGTP is replaced by the same amount of 7-deaza-dGTP in all four dideoxy-NTP solutions.7-Deaza-dGTP lends itself equally well to all other types of dideoxy sequencing, including newly developed double-strand sequencing methods and polymerization techniques. Partial substitution of 7-deaza-dGTP for dGTP in PCR can improve the yield of reaction products for GC-rich templates that contain strong secondary structures. Elimination of spurious GC-hydrogen bonding and relaxation of the secondary structure results in more efficient and specific PCR-product synthesis. Ready-to-use mixtures for the production of RNA transcript, including optimized concentrations of $m^7G(5')ppp(5')G$, are available as Cap Scribe.

For a lab hint on dideoxy DNA sequencing using 7-deaza-dGTP, see section 2.

2. How to Use this Product

Dilute 7-deaza-dGTP into a solution containing 50 mM Tris, pH 7.0, and use at exactly the same concentration as you would use dGTP.

2.1 Procedure

Overcoming Compression Artifacts Using 7-Deaza-dGTP with Klenow or Reverse Transcriptase

One of the most common problems in dideoxy sequencing analysis is the occurrence of "compression" artifacts. Compressions are caused by strong secondary structures in the DNA fragments as they migrate down the sequencing gel. The artifacts appear as abnormal spacing between adjacent bands in a sequencing gel autoradiograph and frequently occur within regions of the DNA template that are rich in G and C residues.

It has been reported that replacement of dGTP in the sequencing reaction mixtures by nucleotide analogs can eliminate most compression artifacts. Substitution with dITP (1) is effective but can result in diminished band intensities (2) and the production of "stop" artifacts (bands across all 4 lanes; reference 3). Recent investigations suggest that substitution with 7-deaza- dGTP is superior to the use of dITP (2,4). Our results concur with this finding, and we routinely use 7-deaza-dGTP in sequencing reactions employing Klenow polymerase or AMV reverse transcriptase.

The recipes listed at right will produce complete dideoxy/deoxynucleotide mixtures ready for use in sequencing experiments using either polymerase. The formulations given are for the use of radiolabeled dATP (20 µCi of 400 Ci/mmol [a-^{32}P]-dATP or 24 −Ci of 600 Ci/mmol [a-^{35}S]-dATP), but can be easily adapted for use with labeled dCTP by substituting dATP for dCTP (and vice versa) in the formulations. These stocks are stable for at least six months if stored at −15 to −25°C.

In a typical set of sequencing reactions, about 0.5–1 µg of annealed template-primer mixture is combined with the labeled nucleotide preparation and the DNA polymerase (either 1 unit of Klenow or 20 U of reverse transcriptase). After thorough mixing, the mixture is divided equally between four tubes, each containing 2.5 µl of the appropriate premixed dideoxy/deoxynucleotide stock. The reactions are incubated at +37 to +42°C for 10 minutes, and then 1 µl of chase mix is added to each tube. Chase mix is composed of 0.5 mM dATP, dCTP, dGTP, and dTTP. After 5 minutes of additional incubation, the reactions are complete.

Although the nucleotide mixtures listed above work well in our hands, modifications may be necessary when sequencing DNA of different base compositions or when using different lots of nucleotide preparations. Lot-to-lot variability in nucleotide preparations can be minimized by using premixed solutions. Should adjustments of the mixtures be necessary, the following rules should apply:

1). If the sequencing reactions (i.e., bands) are more intense at the bottom of the autoradiograph and nearly absent from the top, more of the corresponding deoxynucleotide stock should be added to that reaction mixture.
2). If the bands are more intense at the top and too faint at the bottom of the film, more of the corresponding dideoxynucleotide stock should be added.

Premixed Nucleotides for Klenow Sequencing

Stock solution	A	C	G	T
0.5 mM 7-deaza dGTP (µl)	20	20	1	20
0.5 mM dCTP (µl)	20	2	20	20
0.5 mM dTTP (µl)	20	20	20	1
2 mM ddATP (µl)	5	–	–	–
2 mM ddCTP (µl)	–	5	–	–
2 mM ddGTP (µl)	–	–	6	–
2 mM ddTTP (µl)	–	–	–	25
10× buffer# (µl)	10	10	10	10
H_2O (µl)	25	43	43	24
Total (µl)	100	100	100	100

10× Klenow sequencing buffer = 500 mM Tris-HCl (pH 8.0), 50 mM $MgCl_2$, 300 mM NaCl, 10 mM DTT

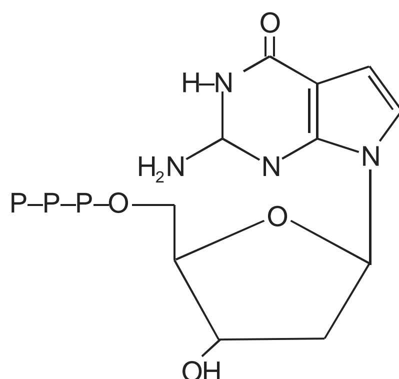
Premixed Nucleotides for Reverse Transcriptase Sequencing

Stock solution	A	C	G	T
5.0 mM 7-deaza-dGTP (μl)	20	20	1	20
5.0 mM dCTP (μl)	20	2	20	20
5.0 mM dTTP (μl)	20	20	20	1
0.5 mM dATP (μl)	2	2	2	2
0.2 mM ddATP (μl)	5	–	–	–
0.2 mM ddCTP (μl)	–	5	–	–
0.2 mM ddGTP (μl)	–	–	6	–
0.2 mM ddTTP (μl)	–	–	–	25
10× buffer## (μl)	10	10	10	10
H_2O (μl)	23	41	41	22
Total (μl)	100	100	100	100

10× AMV reverse transcriptase sequencing buffer = 500 mM Tris-HCl (pH 8.3), 75 mM $MgCl_2$, 400 mM KCl, 10 mM DTT

3. Additional Information on this Product

Formula:
$C_{11}H_{15}N_4O_{13}P_3Li_2$



Molecular weight:
518.1

Purity:
>90% (HPLC)

References

1 Mills, D.R. and Kramer, F.R. (1979) *PNAS, USA* **76**:2232.
2 Barr, P.J. *et al.* (1986) *BioTechniques* **4**:428.
3 Deininger, P.L. (1983) *Anal. Biochem.* **135**:247.
4 Mizusawa, S. *et al.* (1986) *Nucleic Acids Res.* **14**:1319.

4. Suplementary Information

Changes to Previous Version
Editorial changes.

Ordering Information
Roche Applied Science offers a large selection of reagents and systems for life science research. For a complete overview of related products and manuals, please visit and bookmark our home page, www.roche-applied-science.com, and our Special Interest Sites including:

- DNA & RNA preparation - Versatile Tools for Nucleic Acid Purification: http:// www.roche-applied-science.com/napure
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EP Patent 0212536 and US 4,804,748, US 5,480,980, US 5,990,304 owned by Roche Diagnostics GmbH

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