For life science research only.
Not for use in diagnostic procedures.

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

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Lithium salt

Cat. No. 10 988 537 001	2 µmol 200 µl, 10 mM

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	7-Deaza-dGTP, Li-salt	10 mM aqueous solution, pH 7.0.	1 vial, 200 µl

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / bottle	Label	Storage
1	7-Deaza-dGTP, Li-salt	Store at −15 to −25°C.

1.3. Additional Equipment and Reagent required

For preparation of nucleotides for Klenow sequencing

See section, **Working Solution** *for additional information on preparing solutions.*

- Deoxynucleotide Triphosphate Set*
- Dideoxynucleoside Triphosphate Set*
- Tris-HCl*
- $MgCl_2$
- NaCl
- DTT*

For preparation of nucleotides for reverse transcriptase sequencing

See section, **Working Solution** *for additional information on preparing solutions.*

- Deoxynucleotide Triphosphate Set*
- Dideoxynucleoside Triphosphate Set*
- Tris-HCl*
- $MgCl_2$
- KCl
- DTT*

For sequencing reaction using Klenow or reverse transcriptase

- Radiolabeled dATP (20 µCi of 400 Ci/mmol [α-^{32}P]-dATP or 24 µCi of 600 Ci/mmol [α-^{35}S]-dATP)
- Klenow Polymerase*
- AMV Reverse Transcriptase*

1.4. 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 of the DNA. It can be used in a variety of applications:

- The nucleotide 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.

To learn more about dideoxy DNA sequencing using 7-deaza-dGTP, see section, **Protocols**.

2. How to Use this Product

2.1. Before you Begin

General Considerations

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 is effective but can result in diminished band intensities and the production of "stop" artifacts (bands across all 4 lanes). Recent investigations suggest that substitution with 7-Deaza-dGTP is superior to the use of dITP, and should therefore be used in sequencing reactions employing Klenow polymerase* or AMV reverse transcriptase*.

Working Solution

Solution	Composition/Preparation
7-deaza-dGTP, Li-salt	Dilute 7-deaza-dGTP, Li-salt into a solution containing 50 mM Tris, pH 7.0. *Use at exactly the same concentration as you would use dGTP.*
Klenow sequencing buffer, 10x conc.	500 mM Tris-HCl*, pH 8.0, 50 mM $MgCl_2$, 300 mM NaCl, 10 mM DTT*.
AMV reverse transcriptase sequencing buffer	500 mM Tris-HCl*, pH 8.3, 75 mM $MgCl_2$, 400 mM KCl, 10 mM DTT*.
Chase mix	0.5 mM dATP*, dCTP*, dGTP*, and dTTP*.

Premixed nucleotides for Klenow sequencing

Stock Solution	A [µl]	C [µl]	G [µl]	T [µl]	Storage and Stability
0.5 mM 7-Deaza-dGTP	20	20	1	20	–
0.5 mM dCTP	20	2	20	20	Store 6 months at −15 to −25°C.
0.5 mM dTTP	20	20	20	1	
2 mM ddATP	5	–	–	–	
2 mM ddCTP	–	5	–	–	
2 mM ddGTP	–	–	6	–	
2 mM ddTTP	–	–	–	25	
10x Klenow sequencing buffer	10	10	10	10	–
Water	25	43	43	24	–
Total Volume	**100**	**100**	**100**	**100**	

Premixed nucleotides for reverse transcriptase sequencing

Stock Solution	A [µl]	C [µl]	G [µl]	T [µl]	Storage and Stability
5.0 mM 7-Deaza-dGTP	20	20	1	20	–
5.0 mM dCTP	20	2	20	20	Store 6 months at −15 to −25°C.
5.0 mM dTTP	20	20	20	1	
0.5 mM dATP	2	2	2	2	
0.2 mM ddATP	5	–	–	–	
0.2 mM ddCTP	–	5	–	–	
0.2 mM ddGTP	–	–	6	–	
0.2 mM ddTTP	–	–	–	25	
10x AMV reverse transcriptase sequencing buffer	10	10	10	10	–
Water	23	41	41	22	–
Total Volume	**100**	**100**	**100**	**100**	

2.2. Protocols

Sequencing reaction using Klenow or Reverse Transcriptase

See section, **Working Solution** *for additional information on preparing solutions.*

The prepared stock solutions will produce 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 [α-^{32}P]-dATP or 24 µCi of 600 Ci/mmol [α-^{35}S]-dATP), but can be easily adapted for use with labeled dCTP by substituting dATP for dCTP (and vice versa) in the formulations.

1. In a typical set of sequencing reactions, approximately 0.5 to 1 µg of annealed template-primer mixture is combined with the labeled nucleotide preparation and the DNA polymerase, either 1 U of Klenow* or 20 U of Reverse Transcriptase*.

2. After thorough mixing, the mixture is divided equally between four tubes, each containing 2.5 µl of the appropriate premixed dideoxy-/deoxynucleotide stock.

3. The reactions are incubated at +37 to +42°C for 10 minutes, and then 1 µl of Chase mix is added to each tube.
   – After 5 minutes of additional incubation, the reactions are finished.

Although the described nucleotide mixtures work well, 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 apply:

- If the sequencing reactions, that is, the bands are more intense at the bottom of the autoradiograph and nearly absent from the top, add more of the corresponding deoxynucleotide stock to that reaction mixture.
- If the bands are more intense at the top and too faint at the bottom of the film, add more of the corresponding dideoxynucleotide stock.

2.3. Parameters

Chemical Formula

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

Chemical Name

Structural formula

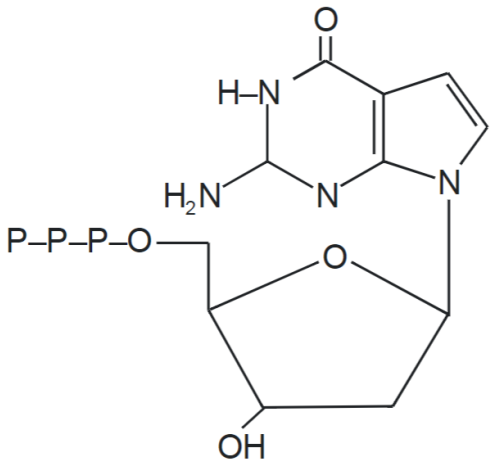

Fig. 1: Structure of 7-Deaza-dGTP, Li-salt.

Molecular Weight

518.1 Da

Purity

>90% (HPLC)

3. Supplementary Information

3.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

3.2. Changes to previous version

Layout changes.
Editorial changes.

3.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
1,4-Dithiothreitol (DTT)	custom fill	10 197 785 103
Tris hydrochloride	500 g	10 812 846 001
Klenow Enzyme	250 U, 5 U/µl	10 104 531 001
Reverse Transcriptase, AMV	1,000 U, > 20 U/µl	10 109 118 001
Deoxynucleoside Triphosphate Set	4 x 250 µl, 4 x 25 µmol, 100 mM	11 969 064 001
	4 x 1,250 µl, 4 x 125 µmol, 100 mM	03 622 614 001
Dideoxynucleoside Triphosphate Set	4 x 1 µmol, 4 x 100 µl, 10 mM	03 732 738 001

3.4. Trademarks

All product names and trademarks are the property of their respective owners.

3.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

3.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

3.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

3.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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