SIGMA-ALDRICH®
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Product Information

57153 ddNTP Set (Dideoxynucleoside Triphosphate Set) Sequencing Grade, sodium salt solutions
4 x 1µmol (4 x 100 µl)

1. Product Overview

Contents
The set contains clear, colorless solutions of dideoxy-nucleotides in water (pH 8.3). ddATP (Cat # 12068), ddCTP (Cat # 02241), ddGTP (Cat # 42414) and ddTTP (Cat # 42597) are each supplied at a concentration of 10 mM.

Product Characteristics
The Dideoxynucleoside Triphosphate Set. Sequencing Grade, sodium salt solution, consists of dideoxynucleotides of high purity (ddNTP HPLC, area % ≥ 98% ddNDP HPLC, area % ≤ 1.5%) specially manufactured and tested for application in sequencing reactions.

Application
2‘,3‘-Dideoxynucleoside triphosphates inhibit the chain elongation of a given primer catalyzed by the DNA polymerase (e.g. Klenow enzyme) and are therefore used for DNA sequencing according to Sanger (1). Sequencing is achieved by including in each reaction a dideoxynucleotide that acts as a chain terminator. Four reactions are set up, each containing the same template and primer but a chain terminator specific for A, C, G or T. Because only a small amount of the chain terminator is included, incorporation into the new DNA strand is a random event. Each reaction therefore generates a collection of fragments, but every DNA strand will end at the same type of base (A, C, G or T).

Storage and Stability
The set is stable at -15 to -25°C through the control date printed on the vial.

Preparation of Termination Mix
Before preparing the termination mix, dilute an appropriate volume of each dideoxynucleotide in water to a concentration of 10 µM. Prepare the termination mix as follows:

1) For maximum recovery of the contents, briefly spin vials in a microcentrifuge before opening.			
2)	To a sterile microfuge tube (on ice) add:		
	Reagent	**Volume**	**Final Concentration**
	dATP, 1 mM	5 µL	100 µM
	dCTP, 1 mM	5 µL	100 µM
	dGTP, 1 mM	5 µL	100 µM
	dTTP, 1 mM	5 µL	100 µM
	ddNTP, 10 µM	2 µL	0.4 µM
	H_2O	28 µL	
	Final volume	50 µL	
3)	Gently vortex the mixture to produce a homogeneous reaction, then centrifuge briefly to collect the sample at the bottom of the tube.		

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2. Quality Control

Function Testing in Sequencing
Each lot of dideoxynucleotide solution is assayed for function in sequencing reaction.

Absence of Contaminating
Deoxyribonucleases/Nicking Activities Each lot of dideoxynucleotide is tested to ensure the absence of deoxyribonucleases (DNases) by incubating increasing amounts of dideoxynucleotide solution in a total volume of 50 µl for 16 h at 37° with 1 µg of EcoR I / Hind III fragments of λDNA or with 1 µg supercoiled plasmid pBR322, respectively. The samples are then subjected to electrophoresis on agarose gel and stained with ethidium bromide. Up to a volume of 20 µl of dideoxynucleotide solution no degradation or changing in the banding pattern is observed, indicating the absence of contaminating DNases/nicking activities.

Absence of Contaminating Ribonucleases
Each lot of dideoxynucleotide is tested to ensure the absence of ribonucleases (RNases) by incubating increasing amounts of dideoxynuoleotide solution for 1 h et 37°C with 4 µl of MS2 RNA. The samples are then subjected to electrophoresis on agarose gel and stained with ethidium bromide. Up to a volume of 20 µl of dideoxynucleotide solution on degradation of the MS2 RNA is observed. indicating the absence of contaminating RNases.

Reference
(1) Sanger, F. et al. (1977) Proc. Natl. Acad. Sci. USA 74, 5463;

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