FT-BS8661

Biotin-X-dCTP

Biotin-X-dCTP can be enzymatically incorporated into DNA via nick translation, random priming, or 3'-end terminal labeling.

Description

Product name cat.number	MW ($g \cdot mol^{-1}$)	Comments
Biotin-dCTP, solution pH 7.5 FP-KA4710, 15 µl (5 mM)	757.51	5-Propargylamino-2'-deoxy-cytidine-5'-triphosphate - Biotin, Triethylammonium salt
Biotin-11-dCTP, solution pH 7.5 FP-BS8661, 15 µl (5 mM)	859.67	γ-[N-(Biotin-6-amino-hexanoyl)]-5-propargylamino-2'-deoxy-cytidine-5'-triphosphate, Triethylammonium salt
Biotin-18-dCTP, solution pH 7.5 FP-DO4100, 15 µl (5 mM)	969.81	γ-[N-(Biotin-6-amino-hexanoyl-6-aminohexanoyl)]-5-(3-propagylamino)-2'-deoxycytidine-5'-triphosphate, Triethylammonium salt

Storage: –20°C >1 year (1 week at room temperature)

Introduction

The number 'X' is the number of atoms in the linker between biotin and dCTP. The length of the linker affects the incorporation efficiency of the biotin-dCTP probe into DNA using DNA polymerases, and it also affects biotin/avidin or biotin/streptavidin. In general, the shorter the linker, the more efficiently the biotin-dCTP is incorporated into DNA by DNA polymerases. On the other hand, the longer the linker, the better biotin can interact with avidin or streptavidin.

Standard protocol

The most popular approach for DNA PCR-labeling with labeled dCTP is based on the usage of dNTPs mixture which contains labeled dCTP and all 4 other dNTP in regular concentrations. The molar ratio dCTP/labeled dCTP can vary from 3 :1 to 1 :1. The incorporation efficiency depends mainly on the usage of dCTP and on the enzyme used for PCR. Regular Taq DNA polymerase incorporates dCTP (and especially labeled dCTP) less efficient than Taq DNA polymerase with modified active center.

In some special applications one may completely substitute dCTP by labeled dCTP and to get DNA with all « C » substituted to labeled dCTP. Meanwhile, this 100% labeled DNA will be quite different from regular DNA in terms of electrophoresis mobility, hydrophobic properties, denaturation behavior etc. If all these points can be neglected , one can completely substitute dCTP by labeled dCTP.

The regular protocol for DNA labeling by PCR use a ratio of 1:2 (labeled dCTP : non-labeled dCTP) :

Reagent	Final concentration	Quantity for 50 µl of reaction mixture
Sterile deionized water	-	Variable
10X PCR buffer	1X	5 µl
10 mM dNTP Mix	0.2 mM of each	1 µl
labeled dCTP, 1 mM	0.1 mM	5 µl
Primer I	0.1 – 1 µM	Variable
Primer II	0.1 – 1 µM	Variable
Taq DNA Polymerase	1.25 U – 2.5 U/50 µl	1.25 U – 2.5 U
100 mM $MgCl_2$	1 – 4 mM	Variable
Template DNA	10 pg – 1 µg	Variable

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PCR should be performed as optimized on the regular dNTPs – with the same $MgCl_2$ concentration, with the temperatures and cycles optimized for the particular template and primers.

Related products

Description	Cat. No.
UptiTherm DNA Polymerase (1000 Units) *5U/µl with Mg free Buffer + 50 mM MgCl2 buffer*	UPS53921
Terminal deoxynucleotidyl transferase (1000 Units)	HP9020
2x Hot Start PCR Master Mix (500 Units)	CJ5361
qPCR Master Mix with EvaGreen (500 Units)	CJ4351
PCR set 1 (dATP, dGTP, dCTP, dTTP 100mM each)	UP968640
PCR Mix 3 (200µl) (10 mM of each dA, dC, dG and dT)	UP984440
GelRed 10000X in water (500 µl)	BY1740

Ordering information

Catalog size quantities and prices may be found at http://www.interchim.com
Please inquire for higher quantities (availability, shipment conditions).

For any information, please ask : FluoProbes® / Interchim; Hotline : +33(0)4 70 03 73 06

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