

Fluorescein-12-dUTP

Fluorescent labelling of DNA by terminal deoxynucleotidyl transferase or DNA-polymerases

Product Information

Name: Fluorescein-12-dUTP,

Fluorescein-5(6)-carboxamidocapoyl-[5(3-aminoallyl)2'-deoxyuridine-5'-

triphosphate)] Tri(triammonium) salt

Catalog Number: FP-BC5471, 30 µl (in water 1 mg/ml)

FP-BC5470, 40 μl (in water 1 mg/ml)

Molecular Weight: MW= 994 (free acid)

Storage: $-20^{\circ}\text{C}_{\text{(J)}}$

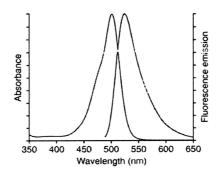
Introduction

Fluorescein-12-dUTP can be incorporated into DNA by terminal deoxynucleotidyl transferase or DNA-polymerases.

The most popular approach for DNA PCR-labeling with Fluorescein-12-dUTP is based on the usage of dNTPs mixture which contains Fluorescein-12-dUTP and all 4 other dNTP in regular concentrations. The molar ratio dUTP/labeled dUTP (or dTTP/labeled dUTP) can vary from 3:1 to 1:1. The incorporation efficiencey depends mainly on the usage of dTTP or dUTP (the incorporation efficiency of dTTP is slightly better than those for dUTP) and on the enzyme used for PCR. Regular Taq DNA polymerase incorporates dUTP (and especially labeled dUTP) less efficient than Taq DNA polymerase with modified active center.

In some special applications one may completely substitute dTTP by Fluorescein-dUTP and to get DNA with all « T » substituted to Fluorescein-12-dUTP. 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 dTTP by Fluorescein-12-dUTP.

Spectra of Fluorescein dUTP in pH 8.0 buffer



FT-BC5470

Directions for use

The regular protocol for DNA – labeling with Fluorescein-12-dUTP by PCR (ratio of labeled dUTP : non-labeled dTTP is 1:2) :

Reagent	Final concentration	Quantity for 50 μl of reaction mixture
Sterile deionized water	-	Variable
10X PCR buffer	1X	5 μ1
10 mM dNTP Mix	0,2 mM of each	1 μ1
Fluorescein-12-dUTP, 1 mM	0,1 mM	5 μ1
Primer I	$0.1 - 1 \mu M$	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

PCR should be performed as optimized on the regular dNTPs – with the same MgCl₂ 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	<u>UPS53921</u>
PCR set 1	dATP, dGTP, dCTP, dTTP 100mM each	<u>UP968640</u>
H1 dialysis membrane	10 mm x 50 cm, 15K MWCO	<u>994920</u>
DAPI, FluoProbes Pure Grade		FP-99963A
FluoProbes 488-dUTP	PCR Grade, 493/517nm	FP-CD0610

References

- **Jon R.I**, *et al* "Apoptotic Responses in Squamous Carcinoma and Epithelial Cells to Small-Molecule Toll-like Receptor Agonists Evaluated with Automated Cytometry", *J Biomol Screen*, **11**: 575 (2006)
- **Rachel L.S**, *et al.*, "Fibroblast Growth Factor-2 inhibits Bleomycin-induced DNA Damage in Murine Lung Endothelial Cells.", *FASEB* J, **20**: A673 (2006)
- **Shang-mian Yie**, *et al.*, "Progesterone regulates HLA-G gene expression through a novel progesterone response element", *Hum. Reprod.*, **21**: 2538 (2006)
- **Van Nguyen T.** *et al.*, DNA damage-induced cellular senescence is sufficient to suppress tumorigenesis: a mouse model, *J. Exp. Med.*, 204: 1453 1461 (2007) <u>Article</u>
- Yabuta N. *et al.*, Lats2 Is an Essential Mitotic Regulator Required for the Coordination of Cell Division, *J. Biol. Chem.*, 282: 19259 19271 (2007) Article

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)470037306

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