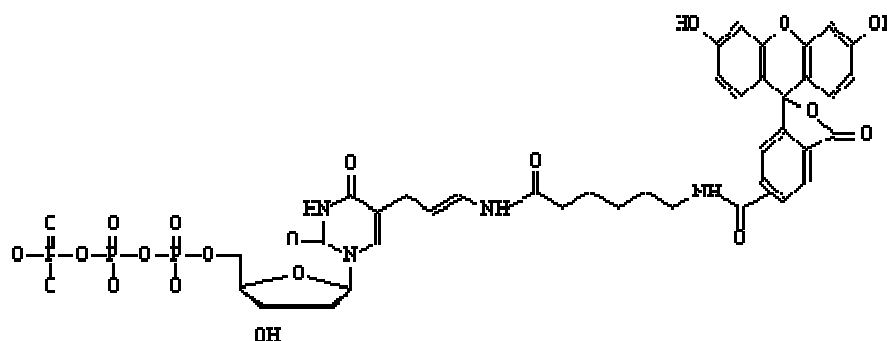


Fluorescein-5(6)-dUTP

Fluorescein-5(6)-carboxamidocabroyl-[5(3-aminoallyl)2'-deoxyuridine-5'-triphosphate, triethylammonium salt

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Product	Cat#	Package size
Fluorescein-5(6)-dUTP (40µL)	M3426.0040	40nmol



Product description

The most popular approach for DNA PCR-labeling with Flu-dUTP or TAMRA-dUTP is based on the usage of dNTPs mixture which contains Flu (TAMRA)-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 efficiency 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. Standard Taq DNA polymerase incorporates dUTP (and especially labeled dUTP) less efficient than dTTP. Sometimes Klenow-Fragment shows better incorporation of modified nucleotides compared to Taq-Polymerase.

In some special applications one may completely substitute dTTP by Flu (TAMRA)-dUTP to get DNA with all "T" substituted to Flu (TAMRA)-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 Flu (TAMRA)-dUTP

Supplied as 1mg/mL solution.

Purity

Higher than 96% (by ion-exchange chromatography, TLC, NMR and UV).

Stability

Fluorescein-12-dUTP is stable for more than 12 months if stored at -20°C. Repeated "freeze-thaw" cycles should be avoided.

Storage

Store after delivery at -20°C. Keep dry and avoid to expose to light sources.

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Protocoll for DNA labelling with Fluorescein-12-dUTP or Tamra-dUTP

Suggested Protocol using Taq Polymerase

Standard protocol for DNA - labeling with Flu (TAMRA)-dUTP by PCR
 (ratio of labeled dUTP:nonlabeled dTTP is 1:2)

Reagent	Final concentration	Quantity for 50 µl reaction
Sterile, de-ionized water	- - -	variable
10X PCR buffer	1X	5µL
10 mM dNTP Mix	0.2 mM each	1µL
Flu (Tamra)-dUTP, 1 mM	0.1 mM	5µL
Primer 1	0.1 - 1.0 µM	variable
Primer 2	0.1 - 1.0 µM	variable
Taq-M Polymerase	1.25 - 2.5 units / 50µL	0.25 - 0.5µL
25 mM MgCl ₂	1 - 4 mM	variable
Template DNA	10pg - 1µg	variable

The PCR reaction should be performed as optimised with standard dNTPs, same MgCl₂ concentration, with the same temperatures and cycles for the particular template and primers.