TexasRed-dUTP

PromoKine

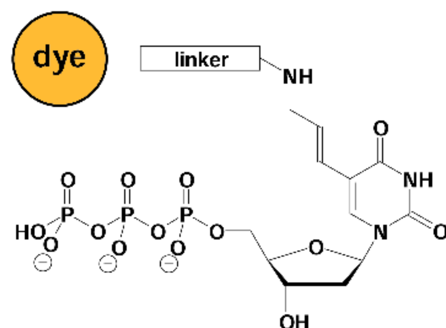
Instruction Manual

Product Name	Product Description	Size	Catalog Number
TexasRed-dUTP	Orange-fluorescent DNA labeling by PCR & Nick Translation	10 µl (1 mM) 50 µl (1 mM)	PK-PFTXR-DUTP-10P PK-PFTXR-DUTP-50P

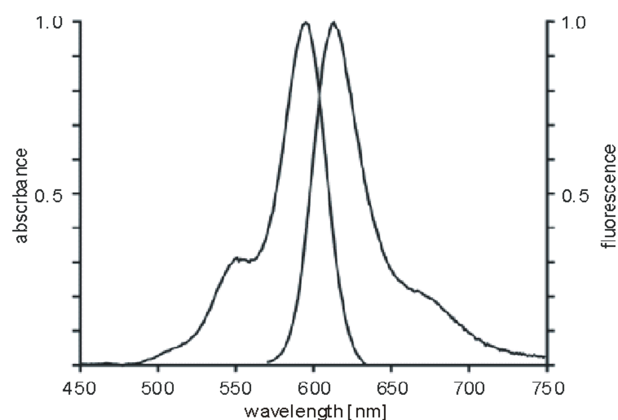
Product Description

TexasRed-dUTP is recommended for direct enzymatic labeling of DNA. The dye-dUTP is specially optimized for incorporation into DNA by PCR or Nick Translation using Taq polymerase or DNA Polymerase I and DNase I. The excellent stability and quantum yield of the fluorophore combined with a high incorporation rate of the dye-dUTP complex makes it the ideal choice for a broad range of fluorescence applications.
In PCR labeling, repeated cycles of denaturation, annealing and extension allow the amplification of a specific DNA fragment. The target DNA is denatured by heating followed by annealing of primers. Extension of the annealed primers with Taq polymerase results in a duplication of the DNA fragment in each cycle. When dTTP is partially substituted by dye-dUTP a fluorescent labeled double-stranded DNA is generated.
Nick translation labeling is based on the reverse activities of Polymerase I and DNase I. DNase I is able to introduce randomly distributed nicks to double stranded DNA at low enzyme concentrations. The 5′→3′ exonuclease activitiy of Polymerase I removes nucleotides from the 3′ side of the nick while synthesizing a partial new complementary strand using the 3′-OH termini as primer. In presence of dye-labeled dUTP the Polymerase I incorporates labeled dUTP instead of dTTP. Well balanced polymerase / nuclease activities in the labeling assay are required for generation of highly labeled double stranded DNA fragments.
The resultant DNA is suited for application in FISH, microarray gene expression profiling and other nucleic acid hybridization assays.
Protect fluorescent labeled dUTP from light and carry out experimental procedures in low light conditions.
Recommended TexasRed-dUTP/dTTP ratio for PCR: 40% TexasRed-dUTP / 60% dTTP. For Nick Translation: 50% TexasRed-dUTP / 50% dTTP.
Note: The optimal final concentration of the Dye-labeled dUTP may strongly depending on the application and assay conditions. For optimal product yields and high incorporation rates an individual optimization of the Dye-labeled-dUTP/dTTP ratio is recommended.

Structure



TexasRed-dUTP, the dye is attached via an optimized linker to aminoallyl-dUTP.



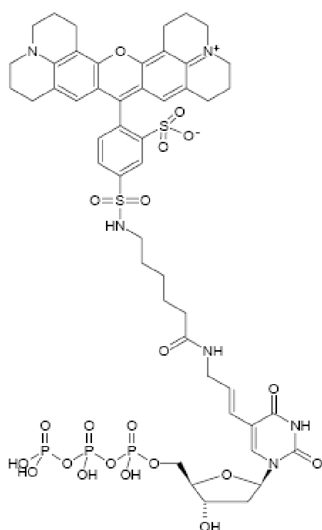
TexasRed excitation and emission spectra.

Spectroscopic data
Excitation maximum: λ_{Ex} = 588 nm
Emission maximum: λ_{Em} = 609 nm
Extinction coefficient: ε_{max} = 80,000 cm^{-1} M^{-1}
$CF_{260} = \varepsilon_{260}/\varepsilon_{max}$ = 0.23
(Tris-HCl, pH 7.5)

Specifications

1 mM clear aqueous solution (pH 7.5) of 5-(3-aminoallyl)-2'-deoxy-uridine-5'-triphosphate labeled with TexasRed, triethylammonium salt
Purity: >95% (as determined by HPLC)
Molecular Formula: $C_{49}H_{59}N_6O_{21}P_3S_2$ (free acid)
Molecular Weight: 1225.07 g/mol (free acid)

Molecular Structure:



Storage and Stability

Store at -20°C in the dark. Avoid frequent thawing and freezing.

Labeling Protocol

Recommended PCR assay

20 µl PCR labeling assay

Component	Stock conc.	Amount	Final conc.
High yield buffer without $MgCl_2$	10x	2 µl	1x
$MgCl_2$ stock solution	25 mM	1.6 µl	2 mM
dATP	1 mM	2 µl	100 µM
dCTP	1 mM	2 µl	100 µM
dGTP	1 mM	2 µl	100 µM
dTTP	1 mM	1.5 µl	75 µM
TexasRed-dUTP	1 mM	0.5 µl [1)]	25 µM [1)]
forward Primer	10 µM	1 µl	500 nM
reverse Primer	10 µM	1 µl	500 nM
Template DNA		0.1-10 ng	5-500 pg/µl
Taq Pol	5 units/µl	0.2 µl (1 unit)	0.05 units/µl
PCR grade H_2O		Fill up to 20 µl	

[1)] The optimal final concentration of the labeled nucleotide may vary depending on the application.

Recommended cycling conditions

Initial denaturation	94°C	2 min	1x
Denaturation	94°C	30 sec	25-30x
Annealing [1)]	50-60°C	30 sec	
Elongation [2)]	72°C	1 min	
Final elongation	72°C	5 min	1x

1. The annealing temperature depends on the melting temperature of the primers used.
2. The elongation time depends on the length of the fragments to be amplified. A time of 2 min/kbp is recommended.

For optimal amplification results and high incorporation rates an individual optimization of the recommended PCR assay and cycling conditions may be necessary for each new primer-template pair.

Recommended NT assay

Sample Material can be supercoiled or linearized plasmid DNA, cosmid or BAC DNA, whole or partial chromosomes or purified PCR products.
Prepare the following reaction mixture in a sterile vial.

20 µl nick translation labeling assay

amount	final conc.	component
2 µl	1x	10x Reaction buffer
1 µl	50 µM	dATP (1 mM)
1 µl	50 µM	dCTP (1 mM)
1 µl	50 µM	dGTP (1 mM)
0.5 µl	25 µM	dTTP (1 mM)
0.5 µl	25 µM	TexasRed-dUTP (1 mM)
1-1.5 µg	50-75 ng/µl	Template DNA
	0.2 u/µl	DNA Polymerase I
	0.002 u/µl	DNase I
fill up to 20 µl		PCR-grade water

1. Vortex the mix gently to assure homogeneity and centrifuge briefly to collect the reaction mixture at the bottom of the tube.
2. Place the tube in a precooled thermomixer at 15°C. The incubation time strongly depends on the Polymerase I / DNase I activities. A well balanced enzyme ratio is required to generate labeled fragments in the desired size range. An individual optimization of the enzyme concentrations is recommended to generate DNA fragments in the range between 200 and 500 bp at an incubation time of 90 minutes.
3. To control the length of the fragments load 2 µl of the assay on an agarose gel. Place the reaction tube at -20°C while running the gel.
4. To get smaller fragments add again Polymerase I and Dnase I and extend the incubation at 15°C.
5. For final stopping the reaction add 5 µl EDTA (0.5 M, pH 8). Proceed to purification of the probe or store at -20°C.

Intended Use

For in vitro research use only. Not for diagnostic or therapeutic procedures.

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