DATA SHEET

Jena Bioscience

HighFidelity ORANGE PCR Labeling Testkit

Preparation of AF594-, ATTO594- and TexasRed-labeled DNA probes by PCR

Cat. No.	Amount
APP-101-ORANGE	1 kit

For general laboratory use.

Shipping: shipped on gel packs

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles, store dark

Shelf Life: 12 months

Spectroscopic Properties:
AF594:
λ_{exc} 590 nm, λ_{em} 617 nm, ε 92.0 L mmol^{-1} cm^{-1} (Tris-HCl pH 7.5)
ATTO594:
λ_{exc} 602 nm, λ_{em} 626 nm, ε 120.0 L mmol^{-1} cm^{-1} (Tris-HCl pH 7.5)
TexasRed:
λ_{exc} 583 nm, λ_{em} 603 nm, ε 112.0 L mmol^{-1} cm^{-1} (Tris-HCl pH 7.5)

Description:
HighFidelity ORANGE PCR Labeling Testkit is designed to produce randomly AF594-, ATTO594- and TexasRed-modified DNA probes by PCR to find the optimal label for the orange emission wavelength range. Such probes are ideally suited for Fluorescence *in situ* hybridization (FISH) and Northern Blot experiments. PCR-based labeling is superior to random-primed labeling with Klenow fragment if template amounts are limited or amplification of a specific DNA fragments is required. Amplification of probes up to 4kbp is feasible.

All labeled-dUTP are efficiently incorporated into DNA as substitute for its natural counterpart dTTP using an optimized reaction buffer and a High Fidelity Polymerase blend consisting of *Taq* polymerase and a proofreading enzyme. 50 % / 25 % labeled-dUTP substitution typically results in an optimal balance between reaction and labeling efficiency. Individual optimization of labeled-dUTP/dTTP ratio however, can easily be achieved with the single nucleotide format.

Content:
High Fidelity Polymerase
in storage buffer with 50% glycerol (v/v)
1x 40 µl (100 units, 2.5 units/µl)

High Fidelity Labeling Buffer
1x 500 µl (10x)

dATP - Solution
1x 20 µl (100 mM)

dGTP - Solution
1x 20 µl (100 mM)

dCTP - Solution
1x 20 µl (100 mM)

dTTP - Solution
1x 20 µl (100 mM)

dUTP-XX-AF594
1x 10 µl (1 mM)

dUTP-XX-ATTO-594
1x 10 µl (1 mM)

dUTP-TexasRed
1x 10 µl (1 mM)

Lambda DNA
1x 20 µl (100 ng/µl)

500 bp forward primer
1x 20 µl (10 µM)

HighFidelity ORANGE PCR Labeling Testkit

Preparation of AF594-, ATTO594- and TexasRed-labeled DNA probes by PCR

500 bp reverse primer
1x 20 µl (10 µM)

PCR-grade water
1x 1.2 ml

To be provided by user
DNA template
Primer
DNA purification tools (optional)

1. Preparation of working solutions

1.1 Preparation of 1 mM dATP/dCTP/dGTP working solution

- Thaw 100 mM dATP, 100 mM dCTP and 100 mM dGTP solutions on ice, voretex and spin-down briefly.
- Prepare a 1:100 dilution with PCR-grade water to achieve a final concentration of 1 mM (e.g. 2 µl 100 mM dATP + 2 µl 100 mM dCTP + 2 µl 100 mM dGTP + 194 µl PCR-grade water).
- 1 mM ATP/CTP/GTP working solution can be stored at -20°C. Prepare aliquots to avoid freeze/thaw cycles.

1.2 Preparation of 1 mM dTTP working solution

- Thaw 100 mM dTTP solution on ice, voretex and spin-down briefly.
- Prepare a 1:100 dilution with PCR-grade water to achieve a final concentration of 1 mM (e.g. 2 µl 100 mM dTTP + 198 µl PCR-grade water).
- 1 mM dTTP working solution can be stored at -20 °C. Prepare aliquots to avoid freeze/thaw cycles.

3. Standard PCR Labeling protocol

The standard protocol is set-up for labeling of a 500 bp DNA fragment. An optimal balance between reaction and labeling efficiency is typically achieved with 50 % dUTP-XX-AF594 & dUTP-XX-594 or with 25 % dUTP-TexasRed substitution following the standard protocol below however, individual optimization might improve results for individual applications.

- Assemble the PCR on ice in the order stated below (DNAse-free reaction tube).
- Voretex and spin-down briefly.
- Perform assay set-up and reaction under low-light conditions.

50 % substitution

Component	Volume	Final concenctration
PCR-grade water	X µl	
High Fidelity Labeling Buffer (10x)	2 µl	1x
1 mM dATP/dCTP/dGTP working solution (s. 1.1)	2 µl	100 µM
1 mM dTTP working solution (s. 1.2)	1 µl	50 µM
1 mM labeled-dUTP	1 µl	50 µM
forward primer (10 µM)	X µl	0.1 - 1 µM (e.g. 0.3 µM 500 bp forward primer)
reverse primer (10 µM)	X µl	0.1 - 1 µM (e.g. 0.3 µM 500 bp reverse primer)
template DNA	X µl	1 - 10 ng genomic DNA (e.g. 1 ng Lambda DNA)
High Fidelity Polymerase (2.5 units/µl)	1 µl	2.5 units
Total volume	20 µl	

HighFidelity ORANGE PCR Labeling Testkit

Preparation of AF594-, ATTO594- and TexasRed-labeled DNA probes by PCR

25 % substitution

Component	Volume	Final concenctration
PCR-grade water	X µl	
High Fidelity Labeling Buffer (10x)	2 µl	1x
1 mM dATP/dCTP/dGTP working solution (s. 1.1)	2 µl	100 µM
1 mM dTTP working solution (s. 1.2)	1.5 µl	50 µM
1 mM labeled-dUTP	0.5 µl	50 µM
forward primer (10 µM)	X µl	0.1 - 1 µM (e.g. 0.3 µM 500 bp forward primer)
reverse primer (10 µM)	X µl	0.1 - 1 µM (e.g. 0.3 µM 500 bp reverse primer)
template DNA	X µl	1 - 10 ng genomic DNA (e.g. 1 ng Lambda DNA)
High Fidelity Polymerase (2.5 units/µl)	1 µl	2.5 units
Total volume	20 µl	

Recommended cycling conditions

Cycle step	Temperature	Time	Cycles
Initial denaturation	95°C	2 min	1x
Denaturation Annealing[1)] Elongation[2)]	95°C 58°C 68°C	20 sec 30 sec 60 sec	30x
Final Elongation	68°C	2 min	1x

[1)]The annealing temperature depends on the melting temperature of primers used.
[2)]The elongation time depends on the length of fragments to be amplified. A time of 2 min/kbp is recommended. Elongation at 72°C works as well.

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.

4. Probe purification:

Probe purification is not required for most hybridization experiments. If a downstream application requires purification (e.g. concentration determination by absorbance measurement) we recommend silica-membrane or gel filtration-based purification.

Related Products:
Aminoallyl-dUTP-XX-AF594, #NU-803-XX-AF594
Aminoallyl-dUTP-XX-ATTO-594, #NU-803-XX-594
Aminoallyl-dUTP-Texas Red, #NU-803-TXR