



## HighFidelity AF594 PCR Labeling Kit

Preparation of AF594-labeled DNA probes by PCR

Cat. No.	Amount
APP-101-AF594-S	10 reactions x 20 µl
APP-101-AF594-L	50 reactions x 20 µl

**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:**  $\lambda_{exc}$  590 nm,  $\lambda_{em}$  617 nm,  $\epsilon$  92.0 L mmol<sup>-1</sup> cm<sup>-1</sup> (Tris-HCl pH 7.5)

### Description:

HighFidelity AF594 PCR Labeling Kit is designed to produce randomly AF594-modified DNA probes by PCR. 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.

dUTP-XX-AF594 is 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 % dUTP-XX-AF594 substitution typically results in an optimal balance between reaction and labeling efficiency. Individual optimization of dUTP-XX-AF594/dTTP ratio however, can easily be achieved with the single nucleotide format.

The kit contains sufficient reagents for 10 labeling reactions (S-Pack) or 50 labeling reactions (L-Pack) of 20 µl each (50% dUTP-XX-AF594 substitution, 100 µM dATP/dGTP/dCTP, 50 µM dTTP, 50 µM dUTP-XX-AF594).

### Content:

#### High Fidelity Polymerase

in storage buffer with 50% glycerol (v/v)

#APP-101-AF594-S: 1x 40 µl (100 units, 2.5 units/µl)

#APP-101-AF594-L: 2x 40 µl (2x 100 units, 2.5 units/µl)

#### High Fidelity Labeling Buffer

1x 500 µl (10x)

#### dATP - Solution

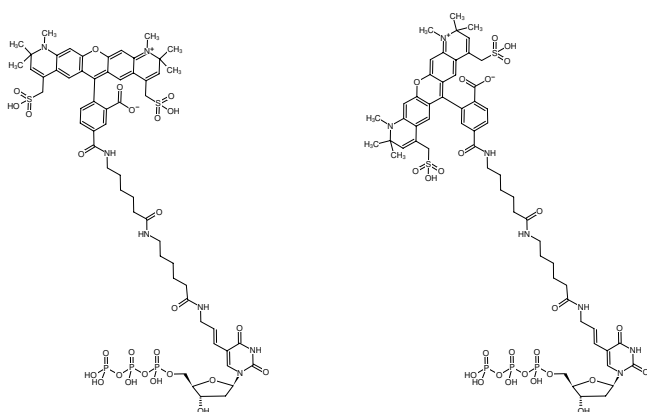
1x 20 µl (100 mM)

#### dGTP - Solution

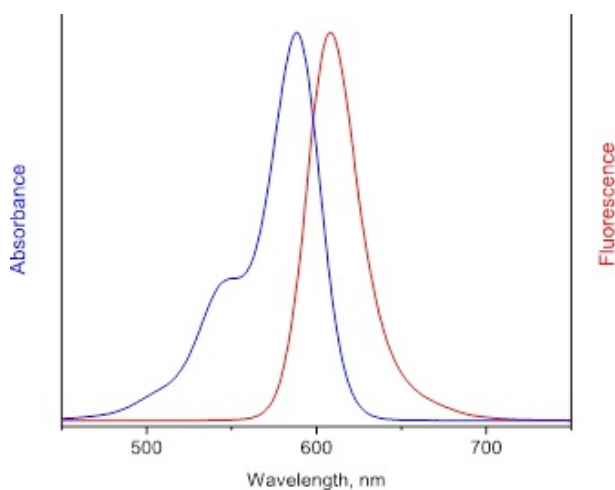
1x 20 µl (100 mM)

#### dCTP - Solution

1x 20 µl (100 mM)



Structural formula of HighFidelity AF594 PCR Labeling Kit



excitation and emission spectrum of AF594



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### dTTP - Solution

1x 20 µl (100 mM)

### dUTP-XX-AF594

#APP-101-AF594-S: 1x 10 µl (1 mM)

#APP-101-AF594-L: 5x 10 µl (1 mM)

### Lambda DNA

1x 20 µl (100 ng/µl)

### 500 bp forward primer

1x 20 µl (10 µM)

### 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, vortex 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, vortex 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 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).
- Vortex and spin-down briefly.
- Perform assay set-up and reaction under low-light conditions.

Component	Volume	Final concentration
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 dUTP-XX-AF594	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	

## Recommended cycling conditions

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

<sup>1)</sup>The annealing temperature depends on the melting temperature of primers used.

<sup>2)</sup>The elongation time depends on the length of fragments to be



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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