



### HighFidelity LNA PCR Labeling Kit

Preparation of LNA-labeled DNA probes by PCR

Cat. No.	Amount
APP-101-LNA	40 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

#### **Description**:

HighFidelity LNA PCR Labeling Kit is designed to produce randomly Locked nucleic acid (LNA)-modified DNA probes by PCR. Such probes are ideally suited for in situ hybridization and Northern Blot experiments.

The ribose ring of locked nucleic acids is "locked" in the ideal conformation for Watson-Crick binding. LNA-modified DNA probes therefore possess an unprecedented thermal stability upon hybridization resulting in an increased sensitivity.

LNA-UTP, LNA-CTP, LNA-ATP and LNA-GTP are efficiently incorporated into DNA as substitute for their natural counterpart (dTTP, dCTP, dATP or dGTP, respectively) using an optimized reaction buffer and a High Fidelity Polymerase. 50 % LNA-NTP substitution typically results in an optimal balance between reaction and labeling efficiency. Individual optimization of LNA-NTP/dNTP ratio however, can easily be achieved with the single nucleotide format.

The kit contains sufficient reagents for 10 labeling reactions each LNA-NTP (20  $\mu$ l each (50 % LNA-NTP substitution).

#### Content:

High Fidelity Polymerase in storage buffer with 50% glycerol (v/v) 2x 40 μl (2x 100 units, 2.5 units/μl)

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

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**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)

**LNA-ATP** 1x 10 μl (1 mM)

**LNA-GTP** 1x 10 μl (1 mM)

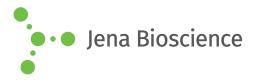
**LNA-CTP** 1x 10 μl (1 mM)

**LNA-UTP** 1x 10 μl (1 mM)



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# **DATA SHEET**





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**Lambda DNA** 1x 20 μl (100 ng/μl)

500 bp forward primer 1x 20  $\mu l$  (10  $\mu M)$ 

**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

Preparation of working solutions is exemplary described for substitution of dTTP by LNA-UTP.

Working solutions for LNA-ATP, LNA-GTP and LNA-CTP are correspondingly prepared by substitution of the natural counterpart (dATP, dGTP or dCTP, respectively)

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% LNA-NTP 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.

Pipetting scheme is exemplary outlined for substitution of dTTP by LNA-UTP:

Component	Volume	Final concenctra- tion	
PCR-grade water	Χ μl		
High Fidelity La- beling Buffer (10x)	2 µl	1x	
1 mM dATP/dCTP/ dGTP working so- lution (s. 1.1)	2 μl	100 µM	
1 mM dTTP working solution (s. 1.2)	1 µl	50 μΜ	
1 mM LNA-UTP	1μl	50 µM	
forward primer (10 µM)	ΧμΙ	0.1 - 1 μM (e.g. 0.3 μM 500 bp forward primer)	
reverse primer (10 μM)	Χ μί	0.1 - 1 μM (e.g. 0.3 μM 500 bp reverse primer)	
template DNA	Χ μί	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		

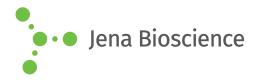
#### **Recommended cycling conditions**

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

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









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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:**

LNA-ATP, #NU-982 LNA-GTP, #NU-983 LNA-CTP, #NU-984 LNA-UTP, #NU-985

