



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 in vitro use only!

Shipping: shipped on blue ice

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 µl 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)

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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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

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, vortext 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, vortext 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).
- Vortex 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 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 LNA-UTP	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 ¹⁾	58°C	30 sec	
Elongation ²⁾	68°C	60 sec	
Final Elongation	68°C	2 min	1x

¹⁾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