DATA SHEET

Jena Bioscience

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 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)

HighFidelity LNA PCR Labeling Kit

Preparation of LNA-labeled DNA probes by PCR

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, 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 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 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 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

HighFidelity LNA PCR Labeling Kit

Preparation of LNA-labeled DNA probes by PCR

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