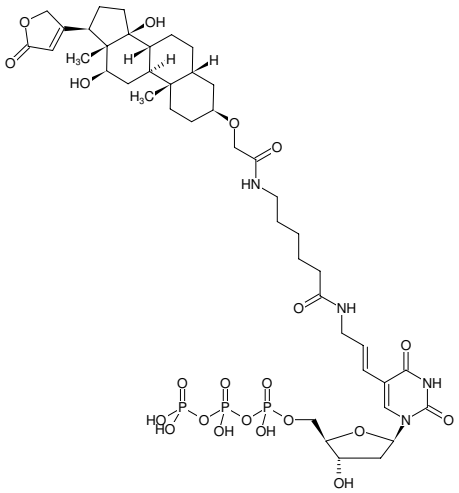
DATA SHEET

Jena Bioscience

HighFidelity Digoxigenin PCR Labeling Kit

Preparation of Digoxigenin-labeled DNA probes by PCR

Cat. No.	Amount
APP-101-DIGX-S	35 reactions x 20 µl
APP-101-DIGX-L	175 reactions x 20 µl



Structural formula of HighFidelity Digoxigenin PCR Labeling Kit

For general laboratory use.

Shipping: shipped on gel packs

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months

Description:
HighFidelity Digoxigenin PCR Labeling Kit is designed to produce randomly Digoxigenin-modified DNA probes by PCR. Such probes are ideally suited for *in situ* hybridization and Northern Blot experiments. PCR-based labeling is superior to random-primed labeling by Klenow fragment if template amounts are limited or amplification of a specific DNA fragments is required.

The labeling principle is similar to the underlying labeling principles of PCR DIG Labeling Mix (Roche) and PCR DIG Probe Synthesis Kit (Roche) with the exception that an alkali-stable version of DIG-11-dUTP is used (DIG label removal not feasible).

DIG-11-dUTP is efficiently incorporated into DNA as substitute for its natural counterpart dTTP using an optimized reaction buffer and a High Fidelity Polymerase. 35 % DIG-11-dUTP substitution typically results in an optimal balance between reaction and labeling efficiency. Individual optimization of DIG-11-dUTP/dTTP ratio however, can easily be achieved with the single nucleotide format. The resulting Digoxigenin-modified DNA probe can subsequently be detected by HRP- or AP-modified Digoxigenin antibody.

The kit contains sufficient reagents for 35 labeling reactions (S-Pack) or 175 labeling reactions (L-Pack) of 20 µl each (35 % DIG-11-dUTP substitution, 100 µM dATP/dGTP/dCTP, 65 µM dTTP, 35 µM DIG-11-dUTP).

Content:
High Fidelity Polymerase
in storage buffer with 50% glycerol (v/v)
#APP-101-DIGX-S: 1x 40 µl (100 units, 2.5 units/µl)
#APP-101-DIGX-L: 1x 200 µl (500 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)

DIG-11-dUTP
#APP-101-DIGX-S: 1x 25 µl (1 mM)
#APP-101-DIGX-L: 5x 25 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

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 35% DIG-11-dUTP 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.

Component	Volume	Final concenctration
PCR-grade water	X µl	
High Fidelity 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)	1.3 µl	65 µM
1 mM DIG-11-dUTP	0.7 µl	35 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 Pol (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.

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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:
DIG-11-dUTP, #NU-803-DIGX