DATA SHEET

Jena Bioscience

Digoxigenin NT Labeling Kit

Cat. No.	Amount
PP-310S-DIGX	10 reactions (20 µl each)
PP-310L-DIGX	75 reactions (20 µl each)

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:
Digoxigenin Nick Translation Labeling Kit contains all reagents (except template and materials for purification of the probe) required for nick translation-based Digoxigenin labeling of DNA providing a highly efficient, easy-to-perform and rapid labeling technology. The labeling principle is similar to the DIG-Nick Translation Mix (Roche).

Nick translation labeling is based on the reverse activities of Polymerase I and DNase I. DNase I is able to introduce randomly distributed nicks to double stranded DNA at low enzyme concentrations. The 5'→3' exonuclease activitiy of Polymerase I removes nucleotides from the 3' side of the nick while synthesizing a partial new complementary strand using the 3'-OH termini as primer. In the presence of labeled dUTP Polymerase I incorporates labeled dUTP instead of dTTP.

The DIGX NT labeling mix contains an optimized Digoxigenin-labeled dUTP for incorporation into DNA by nick translation using DNA Polymerase I.
Digoxigenin-11-dUTP is enzymatically incorporated into DNA as substitute for its natural counterpart dTTP. Optimal substrate properties and thus labeling efficiency as well as an efficient detection of the Digoxigenin moiety is ensured by an 11-atom linker attached to the C5 position of uridine.

The well balanced polymerase / nuclease activities of the enzyme mix ensure the generation of highly labeled double stranded DNA fragments.

The resultant Biotin-labeled DNA is suitable for application in FISH and other nucleic acid hybridization assays.

Content:
Enzyme mix (red cap)
2 units/µl Polymerase I, 0.02 units/µl DNase I in storage buffer

10x NT labeling buffer (green cap)
10x conc.

10x DIGX NT labeling mix (purple cap)
0.5 mM dATP, 0.5 mM dCTP, 0.5 mM dGTP, 0.34 mM dTTP, 0.16 mM Digoxigenin-11-dUTP, pH 7.5

Stop buffer (yellow cap)
0.5 M EDTA, pH 8.0

PCR-grade water (white cap)

Digoxigenin NT Labeling Kit

Recommended NT assay (General Protocol):

Sample material can be supercoiled or linearized plasmid DNA, cosmid or BAC DNA, whole or partial chromosomes or purified PCR products. PCR products should be purified by silica membrane adsorption to remove unincorporated dNTPs (e.g. *PCR Purification Kit, #PP-201*).

Prepare the following reaction mixture in a sterile vial.

20 µl Nick translation labeling assay

2 µl	10x NT labeling buffer	green cap
2 µl	10x DIGX NT labeling mix	purple cap
1-1.5 µg	template DNA	-
2 µl	10x Enzyme mix	red cap
Fill up to 20 µl	PCR-grade water	white cap

- Vortex the mix gently to assure homogeneity and centrifuge briefly to collect the reaction mixture at the bottom of the tube.
- Place the tube in a precooled thermomixer at 15°C. An incubation of 90 min is recommended to generate DNA fragments in a size range between 200 and 500 bp.
- To control the length of the fragments load 2µl of the assay on an agarose gel. Place the reaction tube at -20°C while running the gel.
- To get smaller fragments extend the incubation at 15°C.
- For final stopping of the reaction add 5µl of Stop buffer (yellow cap). Proceed to purification or store at -20°C.

Purification of the probe:
Some downstream applications require the removal of unincorporated dNTPs (labelled and unlabelled). To remove unincorporated nucleotides from the reaction mixture prior to its use in subsequent experiments one of the following procedures is recommended:

1. Purification by silica-gel membrane adsorption - PCR Purification Kit, #PP-201
The Jena Bioscience PCR Purification Kit provides a simple and efficient way to purify DNA fragments larger than 100 bp. The preparation is based on a silica-membrane technology for binding DNA in high-salt and elution in low-salt buffer. Please refer to the instruction manual.

2. Purification by Isopropanol precipitation
Add 1µl glycogene (2 mg/ml), 2 µl sodium acetate (3 M) and 14 µl isopropanol to the reaction mixture and mix well but gently. Incubate on RT for 15 min and spin down at maximum speed at 4°C for 30 min. Discard the supernatant and wash 2x with 70% ethanol (spin down at maximum speed for 5 min).

3. Purification by Centrifugal Filter Units
Unincorporated nucleotides can be removed by centrifugation using centrifugal filter units. Select the filter unit by its cut-off for DNA fragments and follow the manufacturer's instructions.

Related Products:
Digoxigenin-11-dUTP, #NU-803-DIGX