AAT Bioquest®

PRODUCT INFORMATION SHEET

ReadiLink™ DIG (Digoxigenin) Nick Translation dsDNA Labeling Kit

Catalog number: 17472, 17473
Unit size: 10 Reactions, 20 Reactions

Component	Storage	Amount (Cat No. 17472)	Amount (Cat No. 17473)
Component A: DIG-dUTP	Freeze (< -15 °C), Minimize light exposure	1 vial (20 µL)	2 vials (20 µL/vial)
Component B: DNA Polymerase I	Freeze (< -15 °C)	1 vial (10 µL)	2 vials (10 µL/vial)
Component C: DNase I	Freeze (< -15 °C)	1 vial (10 µL)	2 vials (10 µL/vial)
Component D: dNTP mix (dATP, dGTP, dCTP)	Freeze (< -15 °C)	1 vial (50 µL)	2 vials (50 µL/vial)
Component E: dTTP	Freeze (< -15 °C)	1 vial (20 µL)	2 vials (20 µL/vial)
Component F: Nick Translation Buffer	Freeze (< -15 °C)	1 vial (100 µL)	1 vial (100 µL)
Component G: Stop Solution	Freeze (< -15 °C)	1 vial (100 µL)	1 vial (100 µL)

OVERVIEW

ReadiLink™ DIG Nick Translation dsDNA Labelling Kit provides a simple and efficient way to label a double stranded DNA sample with DIG tag. The labelling kit provides all necessary reagents for a complete workflow required for DNA labelling. This method utilizes a combination of DNAse and DNA polymerase to nick one strand of the DNA helix, to which DIG is conjugated. In addition, the kit allows the user to optimize incorporation and product size by adjusting the ratio of DIG-dUTP conjugate to dTTP. It is compatible with a wide variety of sample materials, including bacterial artificial chromosome (BAC) DNA, human genomic DNA, purified PCR products, supercoiled and linearized plasmid DNA. The resulted DIG-labeled DNAs can be used in a variety of molecular biology techniques such as fluorescence in situ hybridization (FISH).

AT A GLANCE

Protocol summary

1. Prepare DNA samples
2. Add reagents to tube
3. Mix and centrifuge briefly
4. Incubate at 15 °C for 60 minutes
5. Place the reaction on ice followed by addition of Stop Solution and heating at 65 °C
6. Place on ice for 5 minutes before using or store at 4 °C
7. Purify the labelled DNA

Important
Thaw all the kit components on ice before starting the experiment. Briefly vortex all the reagents to the bottom before starting the labelling process.

KEY PARAMETERS

Thermal Cycler

Instrument specification(s) N/A

SAMPLE EXPERIMENTAL PROTOCOL

The following protocol can be used as a guideline.
Table 1. Reagents composition per tube for each reaction

Components	Amount
DNA sample	1 µg DNA diluted in Nuclease-free water to final volume of 34 µL
Nick Translation Buffer	5 µL
dNTP mix	5 µL
dTTP	2 µL
DIG-dUTP working solution	2 µL
DNA Polymerase I	1 µL
DNase I	1 µL
Total Volume	**50 µ L**

The ratio of DIG-dUTP (Component A): dTTP (Component E) can be optimized to achieve the best labelling conditions.

Incubation time can be optimized for better labelling. Longer incubation time will help with more labelling but may shorten the size of the end product.

1. To a clean (Nuclease-free) 0.5 mL micro centrifuge tube or 0.2 mL PCR tube, add the reagents in the order indicated in Table 1.
2. Carefully mix the reagents by a brief vortex followed by brief centrifuge.
3. Incubate the reaction at 15 °C for 60 minutes.
4. After incubation, place the reaction on ice.
5. To terminate the reaction, add 5 µL of Stop Solution and heat the sample at 65 °C.
6. Place on ice for 5 minutes before using or store at 4 °C.
7. Purify the labeled DNA.

EXAMPLE DATA ANALYSIS AND FIGURES

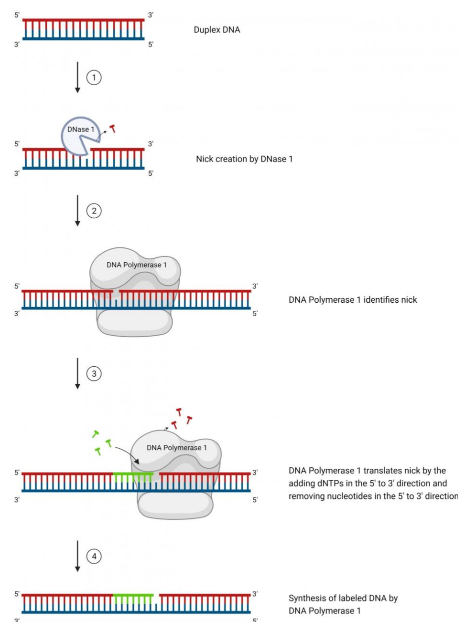

Figure 1. Nick translation labeling of DNA starts with the creation of defects within the sequence of existing DNA double-helix molecules by cleavage of phosphodiester bonds with DNase along the backbone of one strand. Polymerase then repairs these nicks beginning with the removal of the adjacent nucleotide and the immediate filling back in of those gaps with new nucleotides from the added dNTP pool. As each new nucleotide is added, the polymerase

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leaves the 3′ OH group open, thus translating the nick toward the 5′ end. As the reaction sequence is repeated, the polymerase enzyme continues to remove existing nucleotides and replace them with new ones at the site of the new nick. The result of these reactions is numerous labeled and unlabeled nucleotides being incorporated as a complementary sequence along the length of each DNA strand, starting at the site of the original nick.

DISCLAIMER

AAT Bioquest provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please consult the Safety Data Sheet (SDS) provided for the product. Chemical analysis and/or reverse engineering of any kit or its components is strictly prohibited without written permission from AAT Bioquest. Please call 408-733-1055 or email info@aatbio.com if you have any questions.

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