For life science research only.
Not for use in diagnostic procedures.

DIG-Nick Translation Mix

Version: 10
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For generation of highly sensitive probes for *in situ* hybridization labeled with Digoxigenin-11-dUTP.

Cat. No. 11 745 816 910 160 µl
40 labeling reactions

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	DIG-Nick Translation Mix, 5x conc.	▪ Premixed solution for *in situ* probes. ▪ Stabilized reaction buffer in 50% glycerol (v/v) and DNA Polymerase I, DNase I, 0.25 mM dATP, 0.25 mM dCTP, 0.25 mM dGTP, 0.17 mM dTTP, and 0.08 mM DIG-11-dUTP.	1 vial, 160 µl

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	DIG-Nick Translation Mix, 5x conc.	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing. To avoid contamination, aliquot the DIG-Nick Translation Mix solution into 2 to 3 vials.***

1.3. Additional Equipment and Reagent required

For standard labeling reaction

- Heating block
- 0.5 M EDTA, pH 8.0
- Sterile, double-distilled water

For determination of fragment length of labeled probe

- Agarose minigel
- Gel loading buffer
- Heating block
- DNA Molecular Weight Marker*
- 0.5 M EDTA, pH 8.0

1.4. Application

The DIG-Nick Translation Mix is designed for the preparation of probes for *in situ* hybridization applications but can also be used for filter hybridization techniques.

ⓘ *Use DIG-High Prime* for highly sensitive filter hybridization probes. For nonradioactive labeling of in situ probes with other haptens and fluorophores, use the Biotin-Nick Translation Mix* and Nick Translation Mix* (without nucleotides).*

2. How to Use this Product

2.1. Before you Begin

Sample Materials

Templates for labeling reaction

- Supercoiled and linearized plasmid DNA.
- Supercoiled and linearized cosmid DNA.
- Purified PCR products.

i *Denaturing of the template before nick translation is not required.*

General Considerations

Molar ratio

The molar ratio of DIG-11-dUTP to dTTP is adjusted to ensure that every 20th to 25th nucleotide in the newly synthesized DNA is modified with DIG. This density of haptens in the DNA yields the highest sensitivity in the immunological detection reaction.

Labeling of PCR products or varying amounts of template

- When using higher amounts of template DNA (>1 µg) or PCR products as templates, analyze the fragment length distribution of the labeled probe before stopping the reaction with EDTA and heat.
- The total volume of the labeling reaction should be 20 µl/µg template containing 4 µl DIG-Nick Translation Mix/µg template.
- Purify PCR products with the High Pure PCR Product Purification Kit* before nick translation to remove excess unincorporated dNTPs.

Removal of unincorporated free DIG-dNTP

For some *in situ* applications, it might be advantageous to remove unincorporated dNTPs. This can be performed by either ethanol precipitation, gel filtration using Quick Spin Columns for radiolabeled DNA purification, G-50 Sephadex*, or using the High Pure PCR Product Purification Kit*.

Detection of DIG-labeled DNA

DIG-labeled DNA is detected by an antibody conjugated to a fluorophore when metaphase chromosomes or interphase nuclei are used as hybridization targets.
The following conjugates are available:

- Anti-Digoxigenin-Fluorescein, Fab fragments*
- Anti-Digoxigenin-Rhodamine, Fab fragments*

Enhancement of sensitivity

Sensitivity of *in situ* probe detection can be enhanced using an antibody enhancer cascade, such as anti-biotin plus anti-mouse Ig-fluorescein.

Color *in situ* hybridization

For color *in situ* hybridization, for example, for the detection of mRNA or DNA repeats in cellular or tissue preparations, the DIG-labeled DNA is detected by an Anti-Digoxigenin-AP, Fab fragments* conjugate, which catalyzes a color reaction with NBT/BCIP* substrate.

Simultaneous detection of multiple targets

For simultaneous detection of multiple targets in fluorescence *in situ* hybridization, different probes can be labeled with various haptens or fluorophores using the:

- Biotin-Nick Translation Mix* for *in situ* probes.
- Nick Translation Mix* for *in situ* probes.

Working Solution

Solution	Composition	Use	Storage and Stability
EDTA	0.5 M ethylenediaminetetraacetic acid, pH 8.0	Stops the reaction.	+15 to +25°C

2.2. Protocols

Standard labeling reaction

The input DNA can be either linear or supercoiled. Purified PCR products can also be labeled.

1. In a reaction vial, carefully add the following reagents:

Reagent	Volume [µl]
1 µg template DNA in sterile, double-distilled water.	16
DIG-Nick Translation Mix	4

– Mix and centrifuge briefly.

2. Incubate for 90 minutes at +15°C.

3. Stop the reaction by adding 1 µl 0.5 M EDTA, pH 8.0, and heat to +65°C for 10 minutes.

Determination of fragment length of labeled probe

1. After incubation at +15°C, place the reaction on ice.

2. Remove a 3 µl aliquot per 20 µl reaction volume from the reaction, add gel loading buffer, and denature at +95°C for 3 minutes.
   – Place on ice for 3 minutes.
   – Run the sample on an agarose minigel along with a DNA Molecular Weight Marker.
   The probe should range between 200 and 500 nucleotides in length.

3. If necessary, reincubate the reaction at +15°C and check the fragment size again.

4. When correct probe length is achieved, stop the reaction by adding 1 µl 0.5 M EDTA, pH 8.0 per 20 µl reaction volume and heat to +65°C for 10 minutes.

The labeled fragments obtained in the standard labeling reaction show a length distribution maximum in the range of 200 to 500 nucleotides.

3. Additional Information on this Product

3.1. Test Principle

The nick translation method is based on the ability of DNase I to introduce randomly distributed nicks into DNA at low enzyme concentrations in the presence of $MgCl_2$.
E. coli DNA Polymerase I synthesizes DNA complementary to the intact strand in a 5′→3′ direction using the 3′-OH termini of the nick as a primer. The 5′→3′ exonucleolytic activity of DNA polymerase I simultaneously removes nucleotides in the direction of synthesis. The polymerase activity sequentially replaces the removed nucleotides with isotope-labeled or hapten-labeled deoxyribonucleoside triphosphates. At low temperature (+15°C), the unlabeled DNA in the reaction is thus replaced by newly synthesized labeled DNA.

In situ hybridization

In *in situ* hybridization experiments, the fragment length distribution severely influences the efficiency of hybridization. The use of probes showing fragment lengths above the optimal range of 200 to 500 nucleotides usually results in enhanced spotty background signals due to nonspecific sticking of the probe to the glass surface. It also can result in reduced accessibility to the target nucleic acid, such as metaphase chromosomes or cellular and tissue targets. On the other hand, use of excessively short probes will result in poor hybridization efficiency and sensitivity. This is due to fast rehybridization kinetics of short fragments yielding a high proportion of “snapback” probe DNA that reduces the amount of probe being available for hybridization to the target. Thus, the level of DNase I is of high relevance in probe labeling for *in situ* applications.

3.2. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
DNA Molecular Weight Marker VI	50 µg, 1 A_{260}, 200 µl, 50 gel lanes	11 062 590 001
DNA Molecular Weight Marker VIII	50 µg, 1 A_{260}, 200 µl, 50 gel lanes	11 336 045 001
High Pure PCR Product Purification Kit	1 kit, up to 50 purifications	11 732 668 001
	1 kit, up to 250 purifications	11 732 676 001
Quick Spin Columns for radiolabeled DNA purification	20 columns	11 273 965 001
	50 columns	11 273 973 001
DIG-High Prime	160 µl, 40 labeling assays	11 585 606 910
NBT/BCIP Stock Solution	8 ml	11 681 451 001
Nick Translation Mix	200 µl, 50 labeling reactions	11 745 808 910
Biotin-Nick Translation Mix	160 µl, 40 labeling reactions	11 745 824 910
Anti-Digoxigenin-AP, Fab fragments	150 U, 200 µl	11 093 274 910
Anti-Digoxigenin-Fluorescein, Fab fragments	200 µg	11 207 741 910
Anti-Digoxigenin-Rhodamine, Fab fragments	custom fill	11 210 378 103

4.4. Trademarks

HIGH PURE is a trademark of Roche.
All other product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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