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

Nick Translation Kit

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For labeling DNA with radioactive or modified dNTPs.

Cat. No. 10 976 776 001 1 kit
50 labeling assays

Store the kit at −15 to −25°C.

1. General Information 3
1.1. Contents 3
1.2. Storage and Stability 3
Storage Conditions (Product) 3
1.3. Additional Equipment and Reagent required 4
1.4. Application 4

2. How to Use this Product 5
2.1. Before you Begin 5
Sample Materials 5
Templates for labeling reaction 5
General Considerations 5
Labeling efficiency 5
Radioactive dNTP for labeling 5
Working Solution 5
2.2. Protocols 6
Standard radioactive labeling reaction 6
Labeling with Digoxigenin-11-dUTP 6
Biotin-labeled dUTP 6
Removal of unincorporated nucleotides 6
2.3. Parameters 7
Specific Activity 7

3. Results 8
Typical experiment 8
Labeling kinetics 8

4. Additional Information on this Product 9
4.1. Test Principle 9
4.2. Quality Control 9

5. Supplementary Information 10
5.1. Conventions 10
5.2. Changes to previous version 10
5.3. Ordering Information 10
5.4. Trademarks 11
5.5. License Disclaimer 11
5.6. Regulatory Disclaimer 11
5.7. Safety Data Sheet 11
5.8. Contact and Support 11

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	Nick Translation Kit, Control DNA	pBR322 DNA, 50 µg/ml	1 vial, 20 µl
2	Nick Translation Kit, dATP	0.4 mM 2′-deoxyadenosine-5′-triphosphte in Tris buffer.	1 vial, 50 µl
3	Nick Translation Kit, dCTP	0.4 mM 2′-deoxycytidine-5′-triphosphte in Tris buffer.	1 vial, 50 µl
4	Nick Translation Kit, dGTP	0.4 mM 2′-deoxyguanosine-5′-triphosphte in Tris buffer.	1 vial, 50 µl
5	Nick Translation Kit, dTTP	0.4 mM 2′-deoxythymidine-5′-triphosphte in Tris buffer.	1 vial, 50 µl
6	Nick Translation Kit, Buffer, 10x conc.	Nick translation buffer.	1 vial, 100 µl
7	Nick Translation Kit, Enzyme mixture	DNA Polymerase I and DNase I in 50% glycerol (v/v).	1 vial, 100 µl

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / bottle	Label	Storage
1	Control DNA	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
2	dATP	
3	dCTP	
4	dGTP	
5	dTTP	
6	Buffer, 10x conc.	
7	Enzyme mixture	

1.3. Additional Equipment and Reagent required

For standard radioactive labeling reaction

- Heating block
- 0.5 M EDTA, pH 8.0
- Autoclaved, double-distilled water
- [α-^{32}P]dCTP, 3,000 Ci/mmo

For labeling with Digoxigenin-11-dUTP

- Digoxigenin-11-dUTP*, or
- DIG-Nick Translation Mix*
- 0.5 M EDTA, pH 8.0
- Autoclaved, double-distilled water

For biotin-labeled dUTP

- Biotin-16-dUTP*, or
- Biotin-Nick Translation Mix*
- 0.5 M EDTA, pH 8.0
- Autoclaved, double-distilled water

For removal of unincorporated nucleotides

- Quick Spin Columns for radiolabeled DNA purification Sephadex G-50*

1.4. Application

Probes labeled by the Nick Translation Kit are used in many different hybridization techniques.

- Screening gene banks by colony- or plaque hybridization.
- DNA or RNA transfer hybridizations.
- *In situ* hybridization
- Reassociation kinetic studies.

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.

Denaturing of the template before nick translation is not required.

General Considerations

Labeling efficiency

The degree of radioactive labeling is determined by taking an aliquot of the reaction and comparing incorporated to total radioactivity in the aliquot. The kinetics of the reaction may be followed by removing aliquots at various times during the reaction, precipitating the DNA with trichloroacetic acid, and determining the amount of radioactivity in the precipitate.

Radioactive dNTP for labeling

- [α-^{32}P]dCTP is usually used due to its greater stability in comparison to other labeled deoxyribonucleoside triphosphates.
- [α-^{32}P] deoxyribonucleoside triphosphates with a specific activity of 3,000 Ci/mmol give better incorporation and higher levels of labeling than those with a specific activity of 400 Ci/mmol.

Working Solution

Solution	Preparation/Composition	Storage and Stability	For use in...
EDTA	0.5 M ethylenediaminetetraacetic acid, pH 8.0	Store at +15 to +25°C.	Stopping the reaction.
dNTP mixture	▪ Mix 1 volume 0.4 mM Digoxigenin-11-dUTP, 2 volumes dTTP (Vial 5), 3 volumes dATP (Vial 2), 3 volumes dCTP (Vial 3), and 3 volumes dGTP (Vial 4). ▪ The concentration of each of the dNTPs in this mixture is 0.1 mM.	–	Labeling with Digoxigenin-11-dUTP.

2.2. Protocols

Standard radioactive labeling reaction

If the same labeled deoxyribonucleoside triphosphate is used repeatedly, prepare a mixture of equal parts of the other 3 triphosphates. Prepare the dATP, dGTP, dTTP in a 1:1:1 mixture of components 2, 4, and 5.
The input DNA can be either linear or supercoiled. Purified PCR products can also be labeled.
The DNA must be in a low-salt solution.

1. In a reaction vial, add the following components to a microfuge tube on ice.

Reagent	Volume Sample [µl]	Volume Control [µl]
0.1 µg DNA	X	2 (Control DNA, Vial 1)
dATP, dGTP, dTTP mixture	3	3
Buffer, 10x conc. (Vial 6)	2	2
20 µCi (α-^{32}P]dCTP), 3,000 Ci/mmol aqueous solution	2	2
Autoclaved, double-distilled water	add up to a final volume of 18	9
Enzyme mixture (Vial 7)	2	2
Total Volume	**20**	

– Mix and centrifuge briefly.

2. Incubate for 35 minutes at +15°C.

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

Labeling with Digoxigenin-11-dUTP

See section, **Working Solution** *for information on preparing solutions.*
This kit contains nucleotides sufficient for up to 20 DIG-labeling assays. If using the DIG-Nick Translation Mix*, see the Instructions for Use of this product.

1. In a reaction vial, add the following components to a microfuge tube on ice.

Reagent	Volume [µl]
0.1 – 2 µg DNA	X
dNTP mixture	10
Buffer, 10x conc. (Vial 6)	2
Autoclaved, double-distilled water	add up to a final volume of 18
Enzyme mixture (Vial 7)	2

2. Mix and centrifuge briefly.

3. Incubate for 90 minutes at +15°C.

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

Biotin-labeled dUTP

Biotin-labeled dUTP* can be used the same way as Digoxigenin-11-dUTP*. If using the Biotin-Nick Translation Mix*, see the Instructions for Use of this product.

Removal of unincorporated nucleotides

Remove unincorporated dNTPs by either ethanol precipitation or gel filtration using Quick Spin Columns for radiolabeled DNA purification Sephadex G-50*.

2.3. Parameters

Specific Activity

The level of specific labeling and the incorporation rate are dependent on the ratio of substrate DNA to labeled deoxyribonucleoside triphosphate, for example, the kinetics and labeling levels obtained are identical in assays containing 0.1 µg DNA and 20 µCi dXTP or 0.5 µg DNA and 100 µCi dXTP.
The standard assay will give a specific activity of 3×10^8 dpm/µg, corresponding to 65% incorporation with different substrate DNAs, such as pBR322, λDNA, or DNA fragments in 35 minutes, see section, **Results, Figure 1**.

3. Results

Typical experiment

Using the nick translation kit, typical experiments with 0.1 µg λDNA, pBR322 DNA, or 1 kb long pBR322 fragments and 20 µCi α-^{32}P-dCTP, 3,000 Ci/mmol were performed.

- The labeling reaction was monitored at various time points by removal of aliquots and precipitation with trichloroacetic acid, and size analysis of the nick-translated products on denaturing gels.
- After labeling for 15 minutes at +15°C, 65% of the starting material from all 3 DNAs was labeled.
- At maximum labeling levels after 35 minutes, 80% of the fragments in the reactions containing λDNA and pBR322 DNA were 0.2 to 2 kb long.
- After 30 minutes, the reaction with pBR322 restriction fragments resulted in 40% of the fragments maintaining their original length, and with an even distribution of the remaining fragments down to a minimal size of 0.2 kb.
- Fragment size of the nick-translated DNA and also the proportion of snap-back DNA should be considered.
- High levels of DNase I will result in the DNA fragments being excessively short, resulting in poor hybridization reactions. The proportion of snap-back DNA is also increased in this case, reducing the amount of DNA available for hybridization.

Labeling kinetics

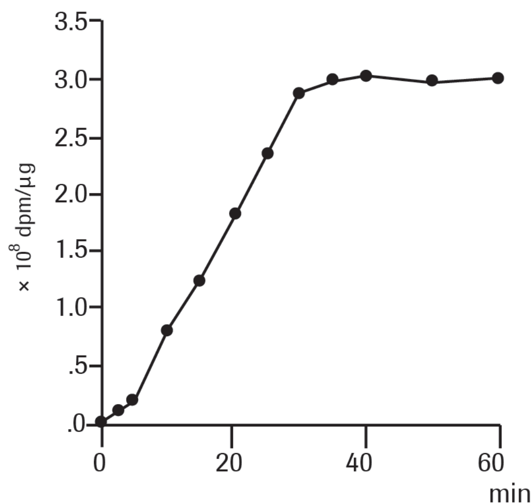

Fig. 1: Labeling kinetics of 0.1 µg DNA with 20 µCi[α-^{32}P]dCTP, 3,000 Ci/mmol.

0.1 µg DNA will be labeled at a constant rate over 70 minutes to a specific activity of 1 × 10^9 dpm/µg, corresponding to 50% incorporation of 100 µCi [α-^{32}P]dCTP, see Figure 2.

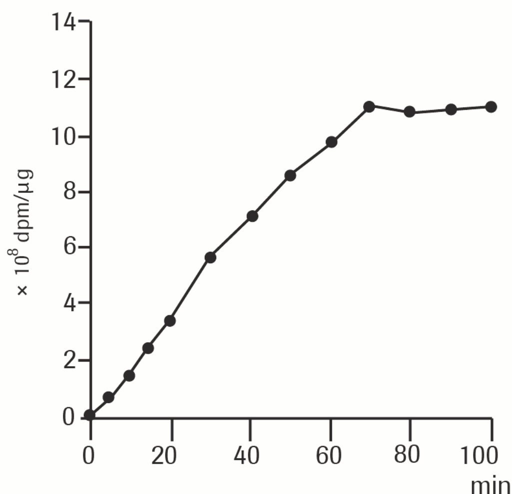

Fig. 2: Labeling kinetics of 0.1 µg DNA with 100 µCi[α-^{32}P]dCTP, 3,000 Ci/mmol.

4. Additional Information on this Product

4.1. 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.

4.2. Quality Control

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

5. Supplementary Information

5.1. 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.

5.2. Changes to previous version

Layout changes.
Editorial changes.

5.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Digoxigenin-11-dUTP, alkali-labile	25 nmol, 25 µl, 1 mM	11 573 152 910
	125 nmol, 125 µl, 1 mM	11 573 179 910
Quick Spin Columns for radiolabeled DNA purification	20 columns	11 273 965 001
	50 columns	11 273 973 001
DIG-Nick Translation Mix	160 µl, 40 labeling reactions	11 745 816 910
Biotin-16-dUTP	custom fill	11 093 711 103
Biotin-Nick Translation Mix	160 µl, 40 labeling reactions	11 745 824 910

5.4. Trademarks

All product names and trademarks are the property of their respective owners.

5.5. License Disclaimer

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

5.6. Regulatory Disclaimer

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

5.7. Safety Data Sheet

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

5.8. 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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