

# Nick Translation Kit

For labeling DNA with radioactive or modified dNTPs

**Cat. No. 10 976 776 001**

Kit for 50 labeling assays

**Version August 2009**  
Store at  $-15$  to  $-25^{\circ}\text{C}$

## 1. What this product does

### Number of Tests

1 kit is sufficient for 50 labeling assays.

### Kit Contents

Bottle	Label	Contents / Function
1	Control DNA	• 20 $\mu\text{l}$ pBR322 DNA (50 $\mu\text{g}/\text{ml}$ )
2	dATP	• 50 $\mu\text{l}$ • [0.4 mM 2'-deoxyadenosine-5'-triphosphate in Tris buffer]
3	dCTP	• 50 $\mu\text{l}$ • [0.4 mM 2'-deoxycytidine-5'-triphosphate in Tris buffer]
4	dGTP	• 50 $\mu\text{l}$ • [0.4 mM 2'-deoxyguanosine-5'-triphosphate in Tris buffer]
5	dTTP	• 50 $\mu\text{l}$ • [0.4 mM 2'-deoxythymidine-5'-triphosphate in Tris buffer]
6	10x Buffer	• 100 $\mu\text{l}$ • [10 $\times$ concentrated nick translation buffer]
7	Enzyme mixture	• 100 $\mu\text{l}$ • enzyme mixture, consisting of DNA-polymerase I and DNase in 50% glycerol (v/v)

### Storage and Stability

The unopened kit is stable at  $-15$  to  $-25^{\circ}\text{C}$  through the expiration date printed on the label.

Avoid repeated freezing and thawing.

### Additional equipment and reagents required

- Heating block
- 0.5 M EDTA (pH 8.0)
- labeled nucleotide (either radioactive or Digoxigenin-linked)

### Application

Probes labeled by nick translation are used in many different hybridization techniques. These include:

- screening gene banks by colony- or plaque hybridization (4),
- DNA or RNA transfer hybridizations (5),
- *in situ* hybridization, and
- reassociation kinetic studies.

## Sample Material

- supercoiled and linearized plasmid DNA
- supercoiled and linearized cosmid DNA
- purified PCR products

Denaturation of the template before nick translation is not required.

## 2. How to use this product

### 2.1 Before you begin

#### DNA

- The DNA must be in low-salt solution.

### 2.2 Procedure for standard (radioactive) labeling

#### dATP, dGTP, dTTP

**Tip:** If the same labeled deoxyribonucleoside triphosphate is used repeatedly, we recommend the preparation of a mixture of equal parts of the other three triphosphates for convenience.

- Prepare the dATP, dGTP, dTTP mixture by making a 1:1:1 mixture of solution 2, solution 4, and solution 5.
- To label the DNA, do the following:

Step	Action																					
1	Add the following to a microfuge tube on ice; the final volume should be 20 $\mu\text{l}$ .																					
	<table border="1"><thead><tr><th>Reagent</th><th>Sample</th><th>Control</th></tr></thead><tbody><tr><td>0.1 <math>\mu\text{g}</math> DNA</td><td>x <math>\mu\text{l}</math></td><td>2 <math>\mu\text{l}</math> control DNA (vial 1)</td></tr><tr><td>dATP, dGTP, dTTP mixture</td><td>3 <math>\mu\text{l}</math></td><td>3 <math>\mu\text{l}</math></td></tr><tr><td>10<math>\times</math>buffer (vial 6)</td><td>2 <math>\mu\text{l}</math></td><td>2 <math>\mu\text{l}</math></td></tr><tr><td>20 <math>\mu\text{Ci}</math> [<math>\alpha</math>-<math>^{32}\text{P}</math>]dCTP, 3000 Ci/mmol aqueous solution</td><td>2 <math>\mu\text{l}</math></td><td>2 <math>\mu\text{l}</math></td></tr><tr><td>Add sterile double dist. water to a final volume of 18 <math>\mu\text{l}</math>.</td><td>x <math>\mu\text{l}</math></td><td>9 <math>\mu\text{l}</math></td></tr><tr><td>Enzyme mixture (vial 7)</td><td>2 <math>\mu\text{l}</math></td><td>2 <math>\mu\text{l}</math></td></tr></tbody></table>	Reagent	Sample	Control	0.1 $\mu\text{g}$ DNA	x $\mu\text{l}$	2 $\mu\text{l}$ control DNA (vial 1)	dATP, dGTP, dTTP mixture	3 $\mu\text{l}$	3 $\mu\text{l}$	10 $\times$ buffer (vial 6)	2 $\mu\text{l}$	2 $\mu\text{l}$	20 $\mu\text{Ci}$ [ $\alpha$ - $^{32}\text{P}$ ]dCTP, 3000 Ci/mmol aqueous solution	2 $\mu\text{l}$	2 $\mu\text{l}$	Add sterile double dist. water to a final volume of 18 $\mu\text{l}$ .	x $\mu\text{l}$	9 $\mu\text{l}$	Enzyme mixture (vial 7)	2 $\mu\text{l}$	2 $\mu\text{l}$
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2	Mix and centrifuge briefly.																					
3	Incubate for 35 min at $15^{\circ}\text{C}$ .																					
4	Stop the reaction by adding 1 $\mu\text{l}$ 0.5 M EDTA (pH 8.0) and/or by heating to $65^{\circ}\text{C}$ for 10 min.																					

## 2.3 Procedure for labeling with Digoxigenin-11-dUTP

### dNTP-Mixture

- Mix 1 vol 0.4 mM Digoxigenin-11-dUTP, 2 vol dTTP (vial 5), 3 vol dATP (vial 2), 3 vol dCTP (vial 3), 3 vol dGTP (vial 4). The concentration of each of the dNTPs in this mixture is 0.1 mM.
- To label the DNA, do the following:

Step	Action	
①	Add the following to a microfuge tube on ice	
	<b>Reagent</b>	<b>Volume</b>
	0.1 µg - 2 µg DNA	× µl
	dNTP mixture	10 µl
	10x buffer (vial 6)	2 µl
	Add sterile double dist. water to a final volume of 18 µl.	×µl
②	Enzyme mixture (vial 7)	2 µl
	Mix and centrifuge briefly.	
③	Incubate for 90 min at 15 °C.	
④	Stop the reaction by adding 1 µl 0.5 M EDTA (pH 8.0) and/or by heating to 65°C for 10 min.	

### Biotin-labeled dUTP

Biotin-labeled dUTP\* can be used the same way as Digoxigenin-11-dUTP.

## 2.4 Removal of unincorporated nucleotides

We recommend removing unincorporated dNTPs by either ethanol precipitation or gel filtration using QuickSpin Columns Sephadex G-50\*.

## 3. Additional Information on this Product

### 3.1 How this Product Works

#### Labeling Principle

The nick translation method (1) is based on the ability of DNase I to introduce randomly distributed nicks into DNA at low enzyme concentrations in the presence of Mg<sup>2+</sup>. *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 (2). The 5' → 3' exonucleolytic activity of DNA polymerase I simultaneously removes nucleotides in the direction of synthesis (3). The polymerase activity sequentially replaces the removed nucleotides with isotope-labeled or hapten-labeled deoxyribonucleoside triphosphates (1). At low temperature (15°C), the unlabeled DNA in the reaction is thus replaced by newly synthesized labeled DNA.

#### 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 [<sup>32</sup>P] dCTP, 3000 Ci/mmol were performed. The labeling reaction was monitored at various time points by removing aliquots, precipitating the DNA with trichloroacetic acid, and determining the size of the nick translated products on denaturing gels.

After 15 min labeling at 15°C, 65% of the starting material from all 3 DNAs was labeled. The label was distributed along the full length of the DNA. At maximum labeling levels (after 35 min), 80% of the fragments in the reactions containing λDNA and pBR322 DNA were 0.2-2 kb long. After 30 min, the reaction with pBR322 restriction fragments resulted in 40% of the fragments maintaining their original length; the remaining fragments were distributed evenly across all sizes, with the smallest fragment 0.2 kb.

Fragment size of the nick translated DNA and also the proportion of "snap-back" DNA should be taken in account. Too much of DNase I will result in the DNA fragments being excessively short and correspondingly poor hybridization reactions. The proportion of snap-back

DNA is also increased in this case, reducing the amount of DNA available for hybridization (6).

## 3.2 Product Characteristics

### Labeling Efficiency

The degree of radioactive labeling is determined by taking an aliquot of the reaction and comparing incorporated radioactivity to total radioactivity in that 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.

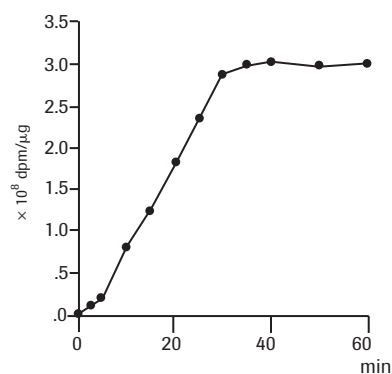
### Radioactive dNTP for labeling

- [<sup>32</sup>P]dCTP is usually used due to its greater stability in comparison to other labeled deoxyribonucleoside triphosphates.
- [<sup>32</sup>P] deoxyribonucleoside triphosphates with a specific activity of 3000 Ci/mmol give better incorporation and higher levels of labeling than those with a specific activity of 400 Ci/mmol.

### Specific Activity

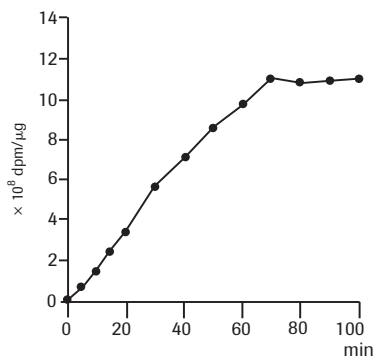
- The level of specific labeling and the incorporation rate are dependent on the ratio of substrate DNA to labeled deoxyribonucleoside triphosphate, e.g. the kinetics and labeling levels obtained are identical in assays containing 0.1 µg DNA and 20 µCi dXTP or 0.5 µg DNA and 100 µCi dXTP.
- The standard assay described here will label different substrate DNAs (e.g. pBR322, λDNA, DNA fragments) to a specificity of 3 × 10<sup>8</sup> dpm/µg, corresponding to 65% incorporation in 35 min (see figure 1).

**Fig. 1:** Labeling kinetics of 0.1 µg DNA with 20 µCi [<sup>32</sup>P]dCTP, 3000 Ci/mmol.



- 0.1 µg DNA will be labeled at a constant rate over 70 min to a specific activity of 1 × 10<sup>9</sup> dpm/µg, corresponding to 50% incorporation of 100 µCi [<sup>32</sup>P] dCTP (see figure 2).

**Fig. 2:** Labeling kinetics of 0.1 µg DNA with 100 µCi [<sup>32</sup>P]dCTP, 3000 Ci/mmol



### Quality Control

Linear kinetics up to 35 min and a specific activity of >2.2 × 10<sup>8</sup> dpm/µg are obtained when the standard assay contains 0.1 µg pBR322 DNA and 20 µCi [<sup>32</sup>P] dCTP, 3000 Ci/mmol. This corresponds to >50% incorporation.

## References

- 1 Rigby, P.W.J. *et al.* (1977) *J. Mol. Biol.* **113**,237-251: Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I.
- 2 Kelly, R.B. *et al.* (1970) *J. Biol. Chem.* **245**, 39-45: Enzymatic synthesis of deoxyribonucleic acid. XXXII. Replication of duplex deoxyribonucleic acid by polymerase at a single strand break.
- 3 Klett, R.P. *et al.* (1968) *Proc. Natl. Acad. Sci. USA* **60**, 943-950: Exonuclease VI, a New Nuclease Activity Associated with *E. coli* DNA Polymerase.
- 4 Grunstein, M. & Hogness, D. (1975) *Proc. Natl. Acad. Sci. USA* **72**, 3961-3965: Colony Hybridization: A Method for the Isolation of Cloned DNAs that Contain a Specific Gene.
- 5 Southern, E. (1975) *J. Mol. Biol.* **98**, 503-517: Detection of specific sequences among DNA fragments separated by gel electrophoresis.
- 6 Norgard, M.V. (1985) *Appl. Biochem. Biotech.* **11**, 1-15: Molecular assessment of S1 endonuclease-resistant snapback hairpin loops generated by DNA polymerase I during the in-vitro nick translation reaction.

Please refer to our website for more information and available printed material:

<http://www.roche-applied-science.com/DIG/>

DIG Product Selection Guide

DIG Application Manual for Filter Hybridization

Non-radioactive *In situ* Hybridization Manual, 3rd ed.

Lab FAQs 3rd ed.

## 4. Supplementary Information

### Changes to Previous Version

Editorial changes

### Text Conventions

To make information consistent and understandable, the following text conventions are used in this Instruction Manual:

Text Convention	Use
Numbered stages labeled ①, ② etc.	Stages in a process that usually occur in the order listed.
Numbered instructions labeled ①, ② etc.	Steps in a procedure that must be performed in the order listed.
Asterisk *	Denotes a product available from Roche Applied Science.

## Contact and Support

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

[www.roche-applied-science.com/support](http://www.roche-applied-science.com/support)

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## Symbols

Symbols are used in this Instruction Manual to highlight important information:

Symbol	Description
	Information Note: Additional information about the current topic or procedure.
	Important Note: Information critical to the success of the procedure or use of the product.

## Ordering Information

For a complete overview of related products, please visit and bookmark our Special Interest Sites including:

- DIG Reagents and Kits for Non-Radioactive Nucleic Acid Labeling and Detection:

<http://www.roche-applied-science.com/DIG>

Product	Pack Size	Cat No.
<b>DIG Kits and Reagents</b>		
Nick Translation Mix for <i>in situ</i> probes	200 µl	11 745 808 910
Anti-Digoxigenin-AP, Fab fragments, from sheep	150 U (200 µl)	11 093 274 910
Anti-Digoxigenin-Fluorescein, Fab fragments, from sheep	200 µg	11 207 741 910
Anti-Digoxigenin-Rhodamine, Fab fragments, from sheep	200 µg	11 207 750 910
High Pure PCR Product Purification Kit	1 kit (250 purifications)	11 732 668 001
Anti-Fluorescein-AP, Fab fragments	150 U	11 426 338 910
HNPP Fluorescent Detection Set	1 set (5 mg HNPP, 100 mg Fast Red)	11 758 888 001
NBT/BCIP stock solution	8 ml	11 681 451 001
NBT/BCIP Ready-to-use tablets	20 tablets	11 697 471 001
Biotin-16-dUTP	50 nmol (50µl)	11 093 070 910
Biotin-High Prime	100 µl (25 reactions)	11 585 649 910
Biotin-Nick Translation Mix for <i>in situ</i> probes	160 µl	11 745 824 910
DIG-11-dUTP alkali-labile	25 nmol (25 µl)	11 573 152 910
DIG-11-dUTP alkali-stable	25 nmol (25 µl)	11 093 088 910
Quick Spin™ Columns, Sephadex® G-50	20 columns	11 273 965 001
Water, PCR Grade	100 ml (4 vials of 25 ml) 25 ml (25 vials of 1 ml) 25 ml (1 vial of 25 ml)	03 315 843 001 03 315 932 001 03 315 959 001
DIG-High Prime	160 µl (40 reactions)	11 585 606 910
DIG-Nick Translation Mix for <i>in situ</i> probes	160 µl	11 745 816 910
Fluorescein-12-dUTP	25 nmol (25 µl)	11 373 242 910
DIG-High Prime	160 µl (40 reactions)	11 585 606 910
Streptavidin-AP	150 U	11 093 266 910
Tetramethyl-rhodamine-6-dUTP	25 nmol (25 µl)	11 534 378 910

## Trademarks

Sephadex is a registered trademark of Pharmacia, Uppsala, Sweden



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Roche Applied Science  
68298 Mannheim  
Germany