For life science research only. Not for use in diagnostic procedures.
FOR *IN VITRO* USE ONLY.

Biotin-Nick Translation Mix

for *in situ* probes
For generation of highly sensitive probes for *in situ* hybridization labeled with biotin-16-dUTP
Premixed solution for 40 labeling reactions

Cat. No. 11 745 824 910

160 µl

Version August 2007

Store at −15 to −25° C

1. Product overview

Contents Sufficient for 40 labeling reactions

Label	Content
Biotin-Nick Translation Mix for *in situ* probes, 5 × conc.	• 160 µl • 5× conc. • 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 Biotin-16-dUTP.

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 $MgCl_2$.

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 (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.

***in situ* hybridization** In *in situ* hybridization experiments, the fragment length distribution influences severely the efficiency of hybridization (4). The use of probes showing fragment lengths above the optimal range of 200-500 nucleotides usually results in enhanced spotty background signals due to unspecific sticking of the probe to the glass surface. It also can result in reduced accessibility to the target nucleic acid (like metaphase chromosomes or cellular and tissue targets). On the other hand use of very 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 (5). Thus the level of DNase I is of high relevance in probe labeling for *in situ* applications. The use of the premixed nick solution reduces pipetting steps and increases the reproducibility of the labeling reaction.

Sample material

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

Note: Denaturation of the template before nick translation is not required.

Application Probes prepared with the Biotin-Nick Translation Mix for *in situ* probes are especially qualified for *in situ* hybridization applications but can also be used for filter hybridization techniques.

Note: For highly sensitive filter hybridization probes we recommend to use Biotin-High Prime*.

For non-radioactive labeling of *in situ* probes with other haptens Roche Applied Science offers the DIG-Nick Translation Mix*.

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

Molar ratio The molar ratio of biotin-16-dUTP to dTTP is adjusted to ensure that every 20th-25th nucleotide in the newly synthesized DNA is modified with biotin This density of haptens in the DNA yields the highest sensitivity in the immunological detection reaction.

Stability The unopened vial is stable at −15 to −25°C through the control date printed on the label.

Note: Repeated freezing and thawing should be avoided. To avoid contamination we recommend to aliquot the Biotin-Nick translation Mix solution and to store in 2-3 portions.

2. Procedures and required material

2.1 Standard labeling reaction

Additional equipment and reagents required

- heating block
- 0.5 M EDTA (pH 8.0)

Procedure In the following table please find a protocol for the standard labeling reaction.

Step	Action
1	Add 1 µg **template DNA** to sterile, double distilled water and end up with a final volume of 16 µl.
2	Add 4 µl **Biotin-Nick Translation Mix,** 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 heating to 65°C for 10 min.

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2.2 Determination of fragment length of the labeled probe

Additional equipment and reagents required

- agarose minigel
- heating block
- DNA molecular weight marker
- 0.5 M EDTA (pH 8.0)

Procedure

Please refer to the following table.

Step	Action
1	After incubation at 15°C, place the reaction on ice.
2	• Take a 3 µl aliquot per 20 µl reaction volume from the reaction. • Add gel loading buffer. • Denature it at 95°C for 3 min and put it on ice for 3 min. • Run the sample on an agarose minigel along with a DNA molecular weight marker. ***Note:*** 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 heating to 65°C for 10 min.

2.3 General remarks on usage and application

Labeling of PCR products or varying amounts of template

- In case of using larger amounts of template DNA (> 1 µg) or PCR products as templates we recommend to 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 Biotin-Nick Translation Mix/µg template.
- PCR products should be purified with the High Pure PCR Product Purification Kit* before nick translation to remove excess unincorporated dNTPs.

Removal of unincorporated free biotin-dNTP

For some *in situ* applications it might be of advantage to remove unincorporated dNTPs. This can be performed by either ethanol precipitation, gel filtration using Quick Spin[1] Columns, Sephadex[2] G-50* or using the *High Pure* PCR Product Purification Kit*.

Detection of biotin-labeled DNA

Biotin-labeled DNA is detected by avidin conjugated to a fluorophore when metaphase chromosomes or interphase nuclei are used as hybridization targets.

Roche Applied Science offers therefore the following conjugates

- avidin-fluorescein,
- avidin-rhodamine

(see ordering information at the end of the pack insert).

Enhancement of sensitivity

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

To further enhance the sensitivity of fluorescence *in situ* hybridization (*e.g.* for detection of single copy targets) we recommend to use streptavidin-AP conjugate in combination with the precipitable fluorescent substrate HNPP/Fast Red* (8).

Color *in situ* hybridization

For color *in situ* hybridization (*e.g.* detection of mRNA or DNA repeats in cellular or tissue preparation) the biotin-labeled DNA is detected by an streptavidin-AP conjugate, which catalyzes a color reaction using NBT/BCIP* substrate (6,7).

Simultaneous detection of multiple targets

For simultaneous detection of multiple targets in fluorescence *in situ* hybridization different probes can be labeled with different haptens or fluorophores using the DIG-Nick Translation Mix for *in situ* probes* and the Nick Translation Mix for *in situ* probes*.

3. Appendix

3.1 References

1 Rigby, P.W.J. et al. (1977) *J. Mol. Biol.* **113**,237-251.
2 Kelly, R.B. et al. (1970) *J. Biol. Chem.* **245,** 39-45.
3 Klett, R.P. et al. (1968) *Proc. Natl. Aced. Sci. USA* **60,** 943-950.
4 Lichter, P. & Cremer, T (1992) in: *Human Cytogenetics - A Practical Approach, Vol. 1*, IRL Press (Rooney, D.E., ad.) 157-192.
5 Norgard, M.V. (1985) *Appi. Biochem. Biotech.* **11**, 1 - 15.
6 Komminoth, P. (1992) *Diagn. Mol. Path.* **1**, 142-150.
7 Komminoth, P. et al. (1992) *Histochemistry* **98,** 217-228.
8 Kagiyama, E. et al. (1993) *Acta Histochem. Cytochem.* **26,** 441-445.

Please refer to our website for the following informations
9 http://www.roche-applied-science.com/DIG/
10 DIG Product Selection Guide
11 DIG Application Manual for Filter Hybridization
12 Non-radioactive In situ Hybridization Manual

3.2 Ordering Information

Kits

Product	Pack Size	Cat. No.
High Pure PCR Product Purification Kit	1 kit (250 purifications)	11 732 668 001
HNPP Fluorescent Detection Set	1 set (5 mg HNPP, 100 mg Fast Red)	11 758 888 001

Single reagents

Product	Pack Size	Cat. No.
Anti-Biotin	100 µg	11 297 597 001
Avidin-Fluorescein	1 mg	11 975 595 910
Avidin- Rhodamine	1 mg	11 975 609 910
Biotin High Prime	100 µl (25 reactions)	11 585 649 910
DIG High Prime	160 µl (40 reactions)	11 585 606 910
DIG Nick Translation Mix for *in situ* probes	160 µl	11 745 816 910
NBT/BCIP Stock Solution	8 ml	11 681 451 001
Nick Translation Mix for *in situ* probes	200 µl	11 745 808 910
Quick Spin Columns, Sephadex G-50	20 columns	11 273 965 001
Streptavidin-AP	150 U	11 093 266 910

* available from Roche Applied Science
[1] Quick Spin is a trademark of a Member of the Roche Group.
[2] Sephadex is a trademark of Pharmacia, Uppsala, Sweden

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The labeling of nucleic acids with DIG is covered by EP patents 0 324 474 and 0 371 262 as well as the following US patents 5.344.757, 5.354.657 and 5.702.888 owned by Roche Diagnostics GmbH.

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