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Nick Translation DNA Labeling System

Instruction Manual

Cat. No. ENZ-42910

50 Reactions

For research use only.

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I. Introduction

Nick translation is recommended for labeling of double stranded DNA larger than 1kb for fluorescent *in situ* hybridization (FISH) applications.

Nick translation is based on the concerted action of *E. coli* DNA Polymerase I and pancreatic DNase I. DNase I introduces random “nicks” in DNA and the DNA Polymerase I catalyzes sequential addition of nucleotide residues to the 3'-OH terminus while simultaneously eliminating nucleotides from the 5'-PO₄ terminus of the nicks. There is no net DNA synthesis. In the presence of fluorophore-labeled nucleotides, pre-existing unmodified nucleotides are replaced by the labeled nucleotides.

The **Nick Translation DNA Labeling System** provides a simple, rapid and efficient method for generating fluorophore-labeled FISH probes. The kit can be used in combination with separately available labeled dNTPs (see list of fluorophore-labeled dUTPs in section IV). All reagents have been optimized for metaphase spreads and interphase nuclei. Additionally, the kit design allows the user the ability to optimize fluorophore incorporation by adjusting the ratio of fluorophore-dUTP and dTTP, and to optimize reaction product size by DNase I digestion.

II. Reagents Provided and Storage

All reagents are shipped on dry ice. Upon receipt, the kit should be stored at ≤-20°C in a non-frost-free freezer. When stored properly, these reagents are stable for at least twelve months. **Avoid repeated freezing and thawing.**

Reagents provided in the kit are sufficient for 50 reactions using 1 µg DNA template per reaction.

Reagent	Vial ID	Volume
DNase I	D	100 µL
10X DNase I Dilution Buffer	DB	1 mL
DNA Polymerase I	DP	250 µL
Reaction Buffer	RB	250 µL
dNTP Mix	dN	250 µL
dTTP	dT	180 µL
Stop Buffer	SB	250 µL
Nuclease-free Water	W	10 mL

III. Additional Materials Required

- Fluorophore-labeled dUTP (see Table 2, section IV)
- Water Baths, Incubators or Thermocyclers set to 15°C, 65°C
- Wet ice or 4°C block.
- Microcentrifuge
- 100 % ethanol
- 70 % ethanol
- 3 M sodium acetate, pH 5.2
- Microcentrifuge
- Nuclease-free TE [10 mM Tris, pH 8.0, 1 mM EDTA]
- Human Cot-1 DNA[®] (Invitrogen, 15279-011)

IV. Related Products

Fluorophore	Reagent	Description	Cat. No.	Ex/Em	Ext. Coeff.
Aqua	Aqua 431 dUTP	DEAC	ENZ-42853	431 / 480	50,000
Green	Green 496 dUTP	5-Fluorescein	ENZ-42831	496 / 520	85,000
	Green 500 dUTP	5-Carboxy-rhodamine Green	ENZ-42845	500 / 522	78,000
Gold	Gold 525 dUTP	5-Carboxy-rhodamine 6G	ENZ-42843	525 / 551	92,000
	Gold 550 dUTP	Enhanced Cyanine 3	ENZ-42521	550 / 576	150,000
Orange	Orange 552 dUTP	5-TAMRA	ENZ-42842	552 / 564	60,000
Red	Red 580 dUTP	5-Rhodamine X (ROX)	ENZ-42844	580 / 603	75,000
	Red 650 dUTP	Enhanced Cyanine 5	ENZ-42522	650 / 662	250,000

* Each vial contains 25 μ L of 1 mM labeled dUTP, enough for at least 25 Nick Translation labeling reactions.

V. Safety Warnings and Precautions

- This product is for research use only and is not intended for use with humans or animals.
- To avoid photobleaching of fluorochrome-labeled nucleotides, perform all manipulations in low light environments, in amber microcentrifuge tubes or protected from light by other means.

VI. Methods and Procedures

A. REAGENT PREPARATION

1. Dilution of Fluorophore

The fluorophore-labeled dUTPs available from Enzo Life Sciences are supplied as a 1mM solution. Dilute to a working concentration of 0.3 mM by adding 10 μ L of labeled dUTP to 23.3 μ L of nuclease-free water. Store the diluted dUTP at -20°C after use.

Nuclease-free Water (W), Reaction Buffer (RB), dNTP Mix (dN), dTTP (dT), Stop Buffer (SB), and 10X DNase I Dilution Buffer (DB) should be thawed at room temperature and then placed on ice. All other reagents should be kept and manipulations performed on ice.

2. Dilution of DNase I

Prepare an ice-cold 1X DNase I Dilution Buffer as needed. Perform a 1:80 dilution of DNase I in 1X Dilution Buffer for both BACs and smaller plasmids.

DNase I should be diluted and added last to the Nick Translation Reaction. **Do not vortex.**

B. NICK TRANSLATION REACTION

1. To a clean microfuge tube, add the reagents in the order indicated in Table 3.

Table 3	
Reagent	Amount
DNA Template (dilute 1 μ g DNA to 25 μ L with Nuclease-free water)	1 μ g DNA in 25 μ L
Reaction Buffer	5 μ L
dNTP Mix	5 μ L
dTTP*	2.5 μ L
Fluorophore-dUTP*	2.5 μ L
DNA Polymerase I	5 μ L
Freshly diluted DNase I	5 μ L
Total Volume	50 μL

* Use of Gold 550 dUTP (Enhanced Cyanine 3) requires 3.3 μ L dTTP and 1.7 μ L of fluorophore-dUTP.

2. Carefully mix the reagents by flicking and briefly centrifuge (~5 seconds).
3. Incubate the reactions for 120 minutes at 15°C.
4. After incubation, place the reactions on ice.
5. Terminate the reaction by adding 5 µL of Stop Buffer and heating for 5 minutes at 65°C.
6. Place on ice for 5 min prior to further use, or store at 4°C.

C. PRECIPITATION OF NICK TRANSLATED PROBES AND PREPARATION OF DNA HYBRIDIZATION MIX

The following procedure describes the precipitation of nick translated DNA probe and subsequent preparation of hybridization mix for use in a single DNA hybridization.

1. Add 5.5 µL (1/10th of nick translation reaction) or desired volume to a fresh microfuge tube. Store remaining probe at -20°C.
2. Add desired amount (12.5-50 ng) of labeled centromeric probe.
3. Add 3 µg Cot-1 DNA.
4. Add nuclease-free water to a total volume of 16 µL.
5. Add 1.6 µL (or 1/10th total volume) of 3 M sodium acetate (pH 5.5).
6. Add 40 µL (or 2.5X total volume) of ice-cold 100% ethanol, mix and place on dry ice for 15 minutes.
7. Pellet the DNA via centrifugation (12,000 rpm for 30 minutes at 4°C).
8. Carefully remove the supernatant and air dry in the dark for at least 15 minutes.
9. Re-suspend the probe in 3 µL of nuclease-free water.
10. Combine the re-suspended pellet with 7 µL of hybridization buffer and proceed with hybridization protocol (see Appendix B).

VII. APPENDICES

A. Recommendations for Labeling DNA Probes

1. Template Quality

The Nick Translation kit was formulated for use with high purity plasmid DNAs (BAC, PACS and smaller DNAs). For optimal results, we recommend DNA samples that are rendered free of RNA, protein, or other contaminants the following procedure:

- a. Treat with RNase A after bacterial lysis.
- b. Use a DNA purification procedure that selectively purifies plasmid DNA from genomic DNA (e.g. anion exchange column-based purification).
- c. Determine purity of the preparation by agarose gel electrophoresis.

2. DNase I Dilution and Analysis of Probe Size

Optimal hybridization results are obtained when probe fragments are between 150 and 500 bp. While most BACs and small plasmids will generate fragment sizes in this range with the suggested 1:80 dilution, it is strongly recommend to analyze the sizes of the reaction products by agarose gel electrophoresis prior to ethanol precipitation.

For agarose gel analysis, remove 3 μL of the reaction, add 1 μL of TE buffer, and 1 μL of 5X Loading Buffer. Heat at 65°C for 5 min. Chill on ice for 2 min and load on a 1.2% to 1.5% agarose gel.

If the product size ranges between 150-500 bp, samples are ready for precipitation. If the product size exceeds 800 bp, repeat the labeling reaction and consider increasing the DNase I concentration (1:70 or 1:60 dilution) or the reaction time (up to 3 hours). If the majority of labeled product is below 100 bp, run another labeling reaction with less amount of DNase I.

B. Recommendations for Hybridization of DNA Probes (Ethanol Precipitated)

1. Denaturation of Probe

- a. Denature the probe by heating the mix for 5 minutes at 73°C in a water bath or a heating block with heated lid.
- b. Place the tube on ice for ~ 2 min. Centrifuge briefly.
- c. Pre-warm the denatured probe mix at 37°C for ≥ 15 minutes before hybridization.

2. Hybridization of Probe(s)

- a. Bring slides to room temperature.
- b. Pre-warm the denaturation buffer (70% formamide, 2x SSC, pH 7.0-8.0) at 75°C for 30 min. Immerse the slides into the hybridization solution for 5 minutes.
- c. Place the slides in 70% ethanol for 1 minute, followed by 85% ethanol for 1 minute and 100% ethanol for 1 minute for dehydration.
- d. Touch the bottom end of the slides to tissue paper sheet to dry the slide. Wipe the underside of the slide with a paper towel.
- e. Place the slide on a slide warmer heated to ~45°C until remnants of ethanol to evaporate.
- f. Apply 10 μ L of the denatured probe (from step 1c) on to the slide.
- g. Apply a clean 22 x 22 mm coverslip and place the slide in a sealed and humidified slide chamber. Incubate at 37°C for 16 hours. Use a solution of 50% formamide, 2X SSC as a humidity control.

3. Post Hybridization Wash

- a. Pre-warm Wash Solution 1 (0.4X SSC, 0.3% NP40) at 73°C for 30 minutes or until cloudy. Add Wash Solution 2 (2X SSC/0.1% NP40) to a separate container and maintain at ambient temperature.
- b. Disassemble the slide chamber, remove the coverslip and place the slide in Wash Solution 1, taking care that the slide does not dry out. Agitate the slide for 1-3 seconds and let stand for 2 minutes at 73°C.
- c. Transfer the slide to container with Wash Solution 2. Agitate for 1-3 seconds and let stand at room temperature for 1 minute.
- d. Dry the slides in the dark.

4. Counterstaining and Mounting

- a. Apply 10 μ L of mounting medium containing DAPI (125 ng/ml) and antifade reagent.
- b. Coverslip each hybridization location.
- c. Visualize under microscope using appropriate fluorescent filter sets.

C. Recommendations for Alternative DNA Probe Purification

For certain applications, removal of unincorporated nucleotides may not be necessary. For applications that require purification of the labeled DNA, we recommend the use of commercially available column filter-based purification systems. To avoid precipitation of reagents or sample, ensure that all solutions are at room temperature and thoroughly mixed before use. All excess wash solution must be removed from the column filter prior to probe elution. Minimize exposure of labeled DNA to light.

Purification by this type of procedure allows determination of yield and dye incorporation. Yield of labeled DNA from the nick translation reaction that is purified by a column-filter based system can range from 250 to 700 ng depending upon the fluorophore-dUTP, DNase I concentration and reaction time. Dye incorporation can vary from 15-60 pmoles depending upon the fluorophore-dUTP used and the ratio of labeled-dUTP and dTTP.

The following protocol is suggested for hybridization of the purified DNA probes:

1. Add 50 ng of purified labeled probe into a fresh microfuge tube.
2. Add 1 μ L (12.5-50 ng) of labeled centromeric probe
3. Add 3 μ g Cot-1DNA.
4. Lyophilize the DNA.
5. Resuspend in 3 μ L of nuclease-free water.
6. Add 7 μ L of hybridization buffer and proceed to hybridization outlined in Appendix B.

VIII. Troubleshooting Guide

Problem	Potential Cause	Suggestion
Low Yield	Poor quality template DNA	Purify the template DNA.
	Overdigestion with DNase I	Use less DNase I.
Probe fragments too long (>800 bp = Punctate background)	Incorrect temperature	Move to calibrated waterbath or thermocycler .
	TE buffer used to dissolve DNA	EDTA inactivates DNase I.
	Not enough DNase I	Increase the amount of DNase I or increase the reaction time.
	Poor quality template DNA	Purify template DNA.
	Inaccurate quantitation	Not enough DNase I, add more DNase I and incubate additional 30 min.
Probe fragments too small (<100 bp = Low signal, diffuse background)	Overdigestion with DNase I	Use less DNase I.
	Inaccurate quantitation due to poor quality template	Purify template DNA.
	Degraded template	Purify template DNA or increase the amount of template DNA in reaction.
High Background	Insufficient Cot-1	Increase amount of Cot-1.
	Too much probe in hybridization	Use less probe .
	Poor quality template DNA	Purify template DNA .
	Inadequate hybridization conditions	Use tightly sealed chamber with appropriate humidity control.
	Insufficient stringency in hybridization or washing	Increase stringency in hybridization buffer or increase stringency of wash solutions, ensure temperatures are correct .
Low Signal	Not enough probe in hybridization	Increase amount of probe in hybridization
	Stringency of hybridization buffer or wash solutions too high	Decrease stringency of hybridization buffer or wash solutions, and ensure that temperatures are correct.
	Inadequate denaturation of slide or probe	Increase denaturation temperature to 74°C.
	Inappropriate filter set on microscope	Multiple bandpass filter sets provide less light than single bandpass. Use correct filter for fluorophore.



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