USER GUIDE

5’ EndTag™ DNA/RNA Labeling Kit

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Cat. No. MB-9001

Storage Store kit at -20 °C upon receipt.

Description

The 5′ EndTag DNA/RNA Labeling Kit facilitates the covalent attachment of a variety of fluorescent dyes, haptens, or affinity tags to the 5′ end of unmodified oligonucleotides or 5′-OH modified DNA or RNA.

The 5′ EndTag Kit is ideal for labeling PCR and sequencing primers because a label is attached only at the 5′ end, leaving the 3′ end available for polymerization. The position of the label does not interfere with hybridization or nucleic acid binding and is, therefore, appropriate for binding of capture probes to affinity matrices and for gel shift assays.

Kit Components†

Product	Volume
5′ EndTag T4 Polynucleotide Kinase	20 µl
5′ EndTag ATPgammaS (ATPγS)	13 µg
5′ EndTag Universal Buffer	30 µl
5′ EndTag Precipitant ††	50 µl
5′ EndTag Alkaline Phosphatase†††	10 µl

One kit contains sufficient reagents for 10 reactions of up to 0.6 nmols of 5′ ends per reaction.

† This kit does not include a thiol-reactive label. Labels may be selected from the table in note F.

†† Precipitant contains purified glycogen and sodium and magnesium salts.

††† Alkaline phosphatase has been included inthe kit to remove the 5′ phosphate group prior to the introduction of the thiophosphate group. Nucleic acids which do not contain a 5′ phosphate, such as unmodified oligonucleotides, require no alkaline phosphatase pretreatment

Reagent Preparation

- Reconstitute ATPγS in 10 µl of nuclease-free, deionized water.
- Reconstitute thiol-reactive label (not included) as described in note F.

Protocol

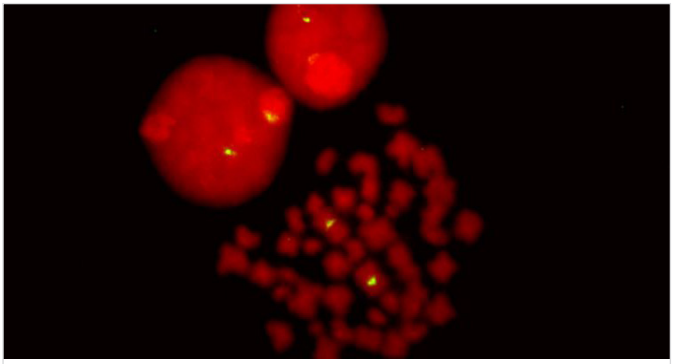
If the nucleic acid contains a 5′ phosphate group, begin at Step 1 (see Note A). If the nucleic acid to be labeled has a 5′ hydroxyl group, begin at Step 2.

1. Combine the following in a microcentrifuge tube:
   -1 µl Universal reaction buffer
   -Nucleic acid (up to 0.6 nmols of 5′ ends in M8 µl; see Note B)
   -1 µl Alkaline phosphatase

   Bring total reaction volume to 10 µl with deionized water. Mix. Incubate for 30 minutes at 37 °C. The entire dephosphorylation reaction mixture can be treated with kinase in Step 2 without purification (see Note C).
2. Combine the following in a microcentrifuge tube (see Notes D and E):
   - 2 µl Universal reaction buffer
   - Nucleic acid (up to 0.6 nmols of 5′ ends in M15 µl; see Note B) or entire reaction mixture from Step 1
   - 1 µl ATPγS
   - 2 µl T4 polynucleotide kinase

   Bring total reaction volume to 20 µl with nuclease-free, deionized water. Mix. Incubate for 30 minutes at 37 °C.
3. Add 10 µl of thiol-reactive label (see Note F). Mix. Incubate for 30 minutes at 65 °C or 2 hours at room temperature.
4. Add 70 µl of nuclease-free water and 100 µl of buffered phenol (see Note G) and vortex briefly. Remove upper aqueous layer to a clean microcentrifuge tube.
5. To this aqueous fraction add 5 µl of precipitant and 270 µl of 95% ethanol. Mix. Pellet the precipitated nucleic acid by centrifugation at 13,000 x g in a microcentrifuge for 30 minutes. Wash the pellet briefly with 70% ethanol and centrifuge at 13,000 x g for 3 minutes. Dry the pellet and resuspend in TE buffer (10 mM Tris, 1 mM EDTA, pH 8).
6. Applications requiring extremely pure nucleic acids (e.g. in situ hybridization) may require additional purification to remove trace amounts of unincorporated label. For these applications, size exclusion chromatography is recommended.

Continued on page 2.



Fluorescence in situ hybridization of human chromosomes using 5’ EndTag Fluorescein-labeled puC1.77 detected directly and mounted in VECTASHIELD® Mounting Media with PI.

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7. Labeling can be confirmed and semiquantitated by comparing dot blots of the 5’ EndTag labeled nucleic acid to those of the labeled control DNA supplied with each maleimide label. Dot 1 ng, 100 pg, 10 pg, and 1 pg of each nucleic acid in 6x SSC (1x = 150 mM NaCl, 15 mM trisodium citrate; pH 7.0) on nitrocellulose or nylon membrane in 1 µl spots. After UV crosslinking, detect by incubating with AP streptavidin (when labeled with biotin maleimide) or with the appropriate AP-labeled antibody and an AP substrate such as BCIP/NBT (SK-5400) or DuoLuX® Chemiluminescent/ Fluorescent Substrate (SK-6605) according to the instructions provided with each product.

Notes

A. Nucleic acids with a 5’ phosphate (e.g. genomic or restriction-digested DNA, in vitro transcribed RNA or decapped mRNA) may be labeled by either of two methods. In the first, the phosphate is left on and labeling is accomplished by the kinase exchange reaction. This method is simplest (begin at Step 2) but results in lower labeling efficiency. The second method involves enzymatic removal of the 5’ phosphate by alkaline phosphatase (Step 1) followed by labeling by the more efficient kinase forward reaction. Unmodified synthetic oligonucleotides typically contain a 5’ hydroxyl group and, therefore, do not require dephosphorylation before end labeling.

B. The concentration of 5’ ends can be estimated by comparison to the examples below. 0.6 nmols of 5’ ends corresponds to:

- 5 µg of 25 base single-stranded or 25 bp double-stranded nucleic acid
- 10 µg of 50 base single-stranded or 50 bp double-stranded nucleic acid
- 20 µg of 100 base single-stranded or 100 bp double-stranded nucleic acid
- 100 µg of 500 base single stranded or 500 bp double-stranded nucleic acid

Alternatively, use the following formula to calculate nmols of 5’ ends in the nucleic acid to be labeled:

$\frac{A}{BxC}$	X = 1000 nmols/µmol = nmols of 5’ ends per µl

where:

A = the concentration of nucleic acid (µg/µl)

B = average molecular weight of nucleotide (333µg/×mol for DNA; 17µg/µmol for RNA)

C = total number of bases (for single-stranded DNA or RNA) or the number of base pairs (for double-stranded DNA).

C. The alkaline phosphatase supplied in this kit will not remove the thiophosphate from the nucleic acid. The AP (and PNK) will be heat inactivated in Step 3 and extracted in Step 4.

D. Ammonium ion concentrations greater than 5 mM and NaCl concentrations greater than 50 mM inhibit PNK activity and will decrease labeling efficiency.

E. For nucleic acids containing recessed 5’ ends, labeling efficiency may be improved by incubating the nucleic acid for 5 minutes at 70 °C immediately followed by chilling on ice for 5 minutes just prior to addition to the PNK reaction in Step 2.

F. The following table shows the available thiol-reactive labels and how they should be dissolved for optimal use with the 5’ EndTag labeling system:

Thiol-reactive label	Cat. No.
Biotin (Long Arm) Maleimide	SP-1501
Fluorescein Maleimide	SP-1502
Texas Red™ Maleimide	SP-1505

G. Buffered phenol can be purchased, or prepared as described in many molecular biology protocol manuals (e.g. Sambrook J, Fritsch EF, et al. Molecular Cloning: A Laboratory Manual, *Cold Spring Harbor Laboratory Press.*

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