

3' EndTag™ DNA Labeling Kit

Cat. No. MB-9002

Storage Store kit at -20 °C upon receipt.

Description The 3' EndTag DNA Labeling Kit enables simple and uniform labeling of the 3' ends of DNA.

3' EndTag-labeled nucleic acids can be used for applications such as DNA hybridization, PCR, *in situ* hybridization and electrophoretic mobility shift assays.

3' EndTag Labeling Reaction Terminal transferase (TdT) catalyzes nucleotide incorporation at the 3' ends of single-stranded or double-stranded DNA. The reaction is template independent although the enzyme prefers single-stranded DNA. The reaction efficiency with double-stranded DNA is significantly improved in the presence of some divalent cations (supplied in the reaction buffer). Overhanging, recessed and blunt 3' ends may all be used as DNA substrates.

The modified nucleotide included in the 3' EndTag Kit is a ribonucleotide. TdT will only incorporate a single ribonucleotide per 3' end, so uniform labeling of multiple probes is assured.

Kit Components[†]

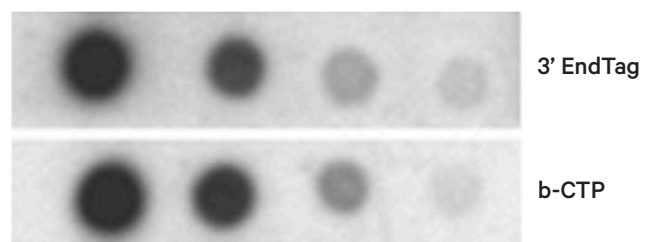
Product	Volume
3' EndTag Terminal Transferase	40 µl
3' EndTag SH-GTP	200 nmoles
3' EndTag 10x TdT Buffer††	40 µl
3' EndTag Precipitant†††	100 µl

One kit contains sufficient reagents for 20 reactions of up to 0.5 nmols of 3' ends per reaction.

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

†† TdT buffer contains sodium cacodylate. Sodium cacodylate is toxic and should be handled with caution.

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



Dot blot hybridization comparing the sensitivity of biotinylated oligonucleotides. The probes were labeled with the 3' EndTag (top) or a terminal transferase reaction with b-CTP (bottom).

Reagent Preparation

- Centrifuge the dry SH-GTP to pellet the powder.
- Reconstitute SH-GTP in 40 µl of deionized water (the resulting concentration is 5 mM).
- Reconstitute thiol-reactive label (not included) as described in note A.

Protocol

This protocol is optimized for labeling of up to 0.5 nmols of 3' ends.

- Combine the following components in a microcentrifuge tube:
 - 0.5 nmols of 3' ends in M 14 µl; see Note B
 - 2 µl 10x TdT reaction buffer
 - 2 µl SH-GTP
 - 2 µl TdT

Bring total reaction volume to 20 µl with deionized water. Mix. Incubate for 30 minutes at 37 °C.

- Add 10 µl of thiol-reactive label (see Note A). Mix. Incubate for 30 minutes at 65 °C.

- Add 70 µl of water and 100 µl of buffered phenol and vortex briefly. Centrifuge for 5 minutes at 4,000 x g. Transfer upper aqueous layer to a clean microcentrifuge tube.

- Add 5 µl of precipitant and 300 µl of 95% ethanol to the aqueous fraction. Mix. Pellet the precipitated nucleic acid by centrifugation

in a microcentrifuge for 15 minutes at 13,000 x g. Wash the pellet briefly with 70% ethanol and centrifuge for 3 minutes at 13,000 x g. Dry the pellet and resuspend in TE buffer (10 mM Tris, 1 mM EDTA, pH 8).

- For applications requiring extremely pure nucleic acids (e.g. *in situ* hybridization) the trace amounts of unincorporated label can be removed by using a standard size exclusion spin column.

See reverse side for additional information.

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Notes

A. The following table lists available thiol-reactive labels and the volume of solvent for preparing working solutions:

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

B. Use the following formula to calculate nmols of 3' ends in the nucleic acid to be labeled:

$\frac{A}{B \times C}$	$X = 1000 \text{ nmols}/\times\text{mol} = \text{nmols of 3' ends per } \mu\text{l}$
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where:

A = the concentration of nucleic acid ($\mu\text{g}/\mu\text{l}$)

B = average molecular weight of DNA nucleotide = $333\mu\text{g}/\mu\text{mol}$

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

Estimating Labeling Efficiency

Successful labeling can be confirmed by comparing the 3' EndTag-labeled nucleic acid to the control labeled DNA (supplied with the thiol-reactive label) in side-by-side dot blotting:

1. Dilute both 3' EndTag-labeled nucleic acid and control labeled DNA to 1 $\mu\text{g}/\text{ml}$, 100 ng/ml , 10 ng/ml , and 1 ng/ml in 6x SSC (900 mM NaCl, 90 mM trisodium citrate, pH 7.0).
2. Dot 1 μl of each dilution on the nitrocellulose or nylon membrane. Crosslink the membrane according to the manufacturer's protocol.
3. Block the membrane in blocking solution (10x Casein solution, Cat. No. SP-5020, can be used).
4. Detect by incubating with AP-streptavidin or appropriate AP-antibody conjugate and an AP substrate such as BCIP/NBT (Cat. No. SK-5400) or DuoLuX® Chemiluminescent/Fluorescent Substrate (Cat. No. SK-6605) according to the instructions provided with the substrate.