DATA SHEET

interchim
Jena Bioscience

3'-End Oligonucleotide Labeling Reagent Kit

Cat. No.	Amount
APP-003	25 reactions x 50 µl (5 pmol each)

For research use only!

Shipping: shipped on blue ice

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months

Description:
3'-End Oligonucleotide Labeling Reagent Kit contains all buffer reagents required for efficient 3'-End Labeling of DNA oligonucleotides (length: 20 -100 bp, 5 pmol per reaction) except of oligonucleotide template to be labeled and labeled nucleotides..

The labeling principle is based on Terminal deoxynucleotidyl Transferase (TdT) that template-independently transfers labeled nucleotides to the 3'-OH group of ssDNA (e.g. an oligonucleotide) in the presence of $CoCl_2$. The number of nucleotide and thus label incorporation depends on the type of nucleotide (UTP/ddUTP) and type of label.

Labeled UTP: 1 – 3 label (average)
Labeled dUTP: multiple label (tail length is highly nucleotide specific)
Labeled ddUTPs: 1 label

The resulting 3'-End labeled oligonucleotides are ideally suited for applications involving sequence-specific protein binding or hybridiziation such as EMSA, Northern or Southern blots. Compared to internal, random labeled probes, the label is located at the 3'-End only and less likely interferes with probe binding.

TdT possesses a preference for single-stranded DNA (ssDNA) over dsDNA with 3'-overhangs or blunt ends. For the preparation of labeled dsDNA complexes, label each complementary oligonucleotide separately and anneal them before use.

Content:
Terminal Deoxynucleotidyl Transferase (TdT)
30 µl (20 U/µl) in 100 mM potassium acetate (pH 6.8), 2 mM 2-mercaptoethanol, 0.01% Triton X-100 (v/v) and 50% glycerol (v/v)

5x TdT Reaction Buffer
400 µl containing 1 M potassium cacodylate, 0.125 M Tris, 0.05% Triton X-100 (v/v), 5 mM $CoCl_2$, pH 7.2

Unlabeled Control Oligonucleotide (60 bp)
250 µl, 1 µM in 1x TE Buffer, pH 7.6

PCR-grade H_2O
12.5 ml

1x TE Buffer, pH 7.6
100 ml containing 10 mM Tris-HCl, 1 mM EDTA, pH 7.6

Stop Buffer
400 µl, 0.5 M EDTA solution, pH 8

3'-End Oligonucleotide Labeling Reagent Kit

1. 3' End Oligonucleotide labeling reaction

- Store all components except of TdT on ice until use.
- Store TdT at -20°C until use.
- Final Assay volume: 50 µl
- Template requirements: oligonucleotide/ssDNA purified by HPLC or gel electrophoresis, 20 – 100 bp
- Add all components on ice exactly in the order listed below.
- Mix reaction gently by pipetting up and down. **Do not voretex!**
- Incubate 30 min at 37 °C.
- Add 1 µl Stop Buffer (0.5 M EDTA solution, pH 8) to stop each reaction.
- Store reactions on ice for subsequent use (see 3.) or -20 °C for long-term storage.

Component	Volume	Final concentration	Final molar amount
PCR grade H_2O	31.5 µl	n/a	n/a
5x TdT Reaction Buffer	10 µl	1x	n/a
oligonucleotide template (1 µM)	5 µl	100 nM	5 pmol
Labeled UTP or ddUTP(10 µM) (see 1.2)	2.5 µl	0.5 µM	50 pmol
TdT (20 U/µl)	1 µl	0.4 U/µl	20 U
Total volume	50 µl		

2. Estimation of Biotin labeling degree

Quantification of labeling degree is essential for reproducible downstream results.
Biotin or Digoxigenin-labeled oligonucleotides can be indirectly detected via Streptavidin or anti-Digoxigenin conjugates, respectively.
The labeling degree of fluorescent oligonucleotides can be directly detected by measurement of the nucleic acid-dye conjugate absorbance followed by a calculation of dye to base ratio according to the law of Lambert-Beer.