DATA SHEET

Jena Bioscience

DIG 3'-End Oligonucleotide Labeling Kit with Digoxigenin-11-ddUTP

Cat. No.	Amount
APP-002	25 reactions x 50 µl (5 pmol each)

For general laboratory use.

Shipping: shipped on gel packs

Storage Conditions: store at -20 °C

Additional Storage Conditions: avoid freeze/thaw cycles

Shelf Life: 12 months

Description:
DIG 3'-End Oligonucleotide Labeling Kit with Digoxigenin-11-ddUTP contains all reagents (except oligonucleotide template to be labeled and materials for Digoxigenin detection) required for efficient 3'-End digoxigenylation of DNA oligonucleotides (length: 20 -100 bp, 5 pmol per reaction).

The labeling principle is based on Terminal deoxynucleotidyl Transferase (TdT) that template-independently transfers 1 Digoxigenin-11-ddUTP to the 3'-OH group of ssDNA (e.g. an oligonucleotide) in the presence of $CoCl_2$. It is similar to the principle of DIG Oligonucleotide 3'-End DNA Labeling Kit, 2nd Generation (Roche).

The resulting 3'-End digoxigenylated oligonucleotides are ideally suited for applications involving sequence-specific protein binding or hybridiziation such as EMSA, Northern or Southern blots. Compared to internal, random digoxigenylated probes, Digoxigenin 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

Digoxigenin-11-ddUTP
25 µl (1 mM)

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

3'-Digoxigenin-labeled Control Oligonucleotide (60 bp)
130 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

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1. Preparation of working solutions

1.1 Preparation of Digoxigenin-11-ddUTP working solution (10 µM)

- Thaw 1 mM Digoxigenin-11-ddUTP solution on ice, voretex and spin-down briefly.
- Prepare a 1: 100 dilution with PCR-grade H2O to achieve a final concentration of 10 µM (e.g. 1 µl of 1 mM Digoxigenin-11-ddUTP + 99 µl PCR-grade H2O).
- Keep working solution (10 µM) on ice until use (see 2.).
- Prepare Digoxigenin-11-ddUTP working solution (10 µM) freshly for each experiment. Do not store for subsequent use.

2. 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
Digoxigenin-11-ddUTP (10 µM) (see 1.1)	2.5 µl	0.5 µM	50 pmol
TdT (20 U/µl)	1 µl	0.4 U/µl	20 U
Total volume	50 µl		

3. Estimation of Digoxigenin labeling degree

Quantification of Digoxigenin labeling degree is essential for reproducible downstream results. An oligonucleotide dilution series is immobilized on a positively-charged membrane (Dot Blot) followed by an indirect detection of the Digoxigenin moiety using an anti-Digoxigenin-alkaline phosphatase (AP) conjugate.

Recommended oligonucleotide starting amount for chemiluminescent detection: 100 fmol
The following reagent amounts (3.1 – 3.5) are calculated for an oligonucleotide starting amount of 100 fmol.

3.1 Preparation of Unlabeled control oligonucleotide (Unlab. oligo) working solution (500 nM)

- Thaw 1 µM unlabeled control oligonucleotide solution on ice, voretex and spin-down briefly.
- Prepare a 1:2 dilution with 1x TE Buffer, pH 7.6 to achieve a final concentration of 500 nM (e.g. 5 µl of 1 µM unlabeled control oligonucleotide + 5 µl 1x TE Buffer, pH 7.6).
- Keep the working solution (500 nM) on ice until use (see 2.).
- Prepare unlabeled control oligonucleotide working solution (500 nM) freshly for each experiment. Do not store for subsequent use.

3.2 Preparation of 3'-Digoxigenin-labeled control oligonucleotide (3'-DIG oligo) working solution (500 nM)

- Thaw 1 µM 3'-Digoxigenin-labeled control oligonucleotide solution on ice, voretex and spin-down briefly.
- Prepare a 1:2 dilution with 1x TE Buffer,pH 7.6 to achieve a final concentration of 500 nM (e.g. 5 µl of 1 µM 3'-Digoxigenin-labeled control oligonucleotide solution + 5 µl 1x TE Buffer, pH 7.6).
- Keep the working solution (500 nM) on ice until use (see 2.)
- Prepare 3'-Digoxigenin-labeled control oligonucleotide working solution (500 nM) freshly for each experiment. Do not store for subsequent use.

3.3 Preparation of Digoxigenin oligonucleotide standard solutions (50 fmol/µl)

- Prepare Digoxigenin oligonucleotide standard solutions (S1 – S5) with varying degrees of oligonucleotide digoxigenylation as follows.
- Total oligonucleotide concentration (S1-S5): 50 fmol/µl
- Degree of dioxygenylation: S1=100 %, S2=75 %, S3= 50 %, S4= 25 %, S5= 0 %
- Voretex and spin-down briefly.

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	S1	S2	S3	S4	S5
3'-DIG oligo (500nM) (s. 3.2)	2 µl	1.5 µl	1 µl	0.5 µl	0 µl
Unlab. oligo (500nM) (s. 3.1)	0 µl	0.5 µl	1 µl	1.5 µl	2 µl
1x TE Buffer pH 7.6	8 µl	8 µl	8 µl	8 µl	8 µl
Total Volume	10 µl	10 µl	10 µl	10 µl	10 µl

3.4 Preparation of sample dilutions (50 fmol/µl)

- Dilute sample labeling reaction(s) (see 1) 1:2 to a final oligonucleotide concentration of 50 nM (e.g. 5 µl sample labeling reaction + 5 µl 1x TE Buffer, pH 7.6.)
- Voretex and spin-down briefly.

3.5 Preparation oligonucleotide standard and sample dilution series

- Transfer 10 µl of each Digoxigenin oligonucleotide standard solution S1 – S5 (see 2.1) to well A1 – A5 of a low absorption 96-well PCR plate, respectively (e.g. 96-well Multiply® PCR plate, Sarstedt, #72.1979.102).
- Transfer 10 µl of sample dilution(s) (see 2.2) to the remaining A" wells (A6 to A...)
- Prepare a two-fold dilution series with 1x TE Buffer as follows:

	1	2	3	4	5	6
A	10µl of S1	10µl of S2	10µl of S3	10µl of S4	10µl of S5	10µl sample
B	5µl A1 + 5µl TE	5µl A2 + 5µl TE	5µl A3 + 5µl TE	5µl A4 + 5µl TE	5µl A5 + 5µl TE	5µl A6 + 5µl TE
C	5µl B1 + 5µl TE	5µl B2 + 5µl TE	5µl B3 + 5µl TE	5µl B4 + 5µl TE	5µl B5 + 5µl TE	5µl B6 + 5µl TE
D	5µl C1 + 5µl TE	5µl C2 + 5µl TE	5µl C3 + 5µl TE	5µl C4 + 5µl TE	5µl C5 + 5µl TE	5µl C6 + 5µl TE
E	5µl D1 + 5µl TE	5µl D2 + 5µl TE	5µl D3 + 5µl TE	5µl D4 + 5µl TE	5µl D5 + 5µl TE	5µl D6 + 5µl TE
F	5µl E1 + 5µl TE	5µl E2 + 5µl TE	5µl E3 + 5µl TE	5µl E4 + 5µl TE	5µl E5 + 5µl TE	5µl E6 + 5µl TE

3.6 Dot Blot and UV crosslinking

- Equilibrate a positively-charged membrane of appropriate size for at least 10 minutes in 1x TE Buffer, pH 7.6 (e.g. Biorad Zeta-Probe® Membrane, #1620159).
- Place the equilibrated membrane onto a clean dry Whatman® paper. Allow excess buffer to absorb into the membrane, but do not let the membrane dry out.
- Spot 2 µl of each dilution onto the membrane.

3'-OH [fmol]	100% DIG	75% DIG	50% DIG	25% DIG	0% DIG	Sample
100	2µl A1	2µl A2	...	...	...	2µl A6
50	2µl B1	...				...
25	...					
12.5						
6.25						
3.125						

- Allow samples to absorb into the membrane.
- Immediately fix the oligonucleotide to the membrane by crosslinking with UV-light using a commercial UV-light crosslinking instrument according to the manufacturers instructions (e.g. 120 mJ/cm^2, 254 nm bulbs, 45-60 second exposure).
- Proceed immediately with detection (see 3.7) or store the membrane dry at room-temperature.

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3.7 Digoxigenin detection with anti-Digoxigenin-Alkaline Phosphatase (AP)

- Perform Digoxigenin detection with an appropriate anti-Digoxigenin-alkaline phosphatase (AP) conjugate followed by chemiluminescent.
- Compare spot intensities of sample lanes to those of control oligonucleotide template and Digoxigenin oligonucleotide standard.
  Please note: Digoxigenin labeling degree may vary depending on the template (e.g. purity, length or overall sequence).

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