


VII. Oligonucleotide tailing with a DIG-dUTP, Biotin-dUTP, or Fluorescein-dUTP mixture

Terminal Transferase is used to add a mixture of labeled dUTP and dATP to the 3' ends of an oligonucleotide in a template-independent reaction (Schmitz et al., 1990). In the tailing reaction, the concentrations of labeled dUTP and unlabeled dATP are adjusted to produce the highest hapten incorporation, optimal spacing of the hapten, and (ultimately) the highest sensitivity.


This procedure produces a tail which ranges in length from 10–100 nucleotides (average: 50), and contains, on average, 5 labeled dUTP molecules. Alternatively, this procedure may be modified to add only 2–3 labeled dUTP molecules, without any intervening dATP. (For details on modifying the length and composition of the tail, see the pack insert from the DIG Oligonucleotide Tailing Kit).

 *HPLC- or gel-purified oligonucleotides from 14–100 nucleotides long can be labeled in this procedure.*

1 Dissolve the purified oligonucleotide in sterile double dist. water.

2 Prepare a 1 mM solution of X-dUTP (X = DIG, Biotin, or Fluorescein) in double dist. water.

3 Add the following to a microcentrifuge tube on ice:


 *The 5× reaction buffer and the Terminal Transferase contain toxic material. Handle with care.*

▶ 4 µl of 5× concentrated reaction buffer [1 M potassium cacodylate, 0.125 M Tris-HCl, 1.25 mg/ml Bovine Serum Albumin; pH 6.6 (25°C)] (vial 1)

 *Numbered vials are included in the DIG Oligonucleotide Tailing Kit.*

▶ 4 µl of 25 mM CoCl₂ (vial 2).

▶ 100 pmol oligonucleotide.

 *Do not increase the concentration of oligonucleotide in this standard reaction. To make larger amounts of labeled oligonucleotide, scale up all reaction components and volumes.*

▶ 1 µl of either 1 mM DIG-dUTP (vial 3) or 1 mM Biotin-dUTP or 1 mM Fluorescein-dUTP.

▶ 1 µl of 10 mM dATP [in Tris buffer, pH 7.5 (25°C)] (vial 4).

▶ 1 µl (50 U) Terminal Transferase [supplied in 200 mM potassium cacodylate, 1 mM EDTA, 200 mM KCl, 0.2 mg/ml Bovine Serum Albumin, 50% glycerol; pH 6.5 (25°C)] (vial 5).

▶ Add double dist. water to a final volume of 20 µl.


4 Mix the reaction components well and centrifuge briefly.

5 Incubate at 37°C for 15 min, then place on ice.

6 **Optional:** Stop the reaction by doing the following:

▶ Mix 200 µl 0.2 M EDTA (pH 8.0) with 1 µl of glycogen solution (20 mg/ml, double dist. water) (vial 9).

▶ Add 2 µl of the glycogen-EDTA mixture to the reaction mixture.

 *Do not use phenol/CHCl₃ extraction to stop the reaction, since the labeled oligonucleotide will migrate to the organic layer during such extraction.*




7 **Optional:** Precipitate the labeled oligonucleotide by performing the following steps:

- ▶ To the reaction tube, add 2.5 μ l 4 M LiCl and 75 μ l prechilled (-15 to -25°C) 100% ethanol. Mix well.
- ▶ Let the precipitate form for at least 30 min at -70°C or 2 h at -15 to -25°C.
- ▶ Centrifuge the tube (at 13,000 \times *g*) for 15 min at 2–8°C.
- ▶ Discard the supernatant.
- ▶ Wash the pellet with 50 μ l ice-cold 70% (v/v) ethanol.
- ▶ Centrifuge the tube (at 13,000 \times *g*) for 5 min at 2–8°C.
- ▶ Discard the supernatant.
- ▶ Dry the pellet under vacuum.

8 Do one of the following:


- ▶ *If you are not going to use the labeled oligonucleotide probe immediately, dissolve the pellet in a minimal amount of sterile, double dist. water and store the probe solution at -15 to -25°C.*

 *Avoid repeated freezing and thawing of the probe.*

- ▶ *If you are going to use the labeled oligonucleotide probe immediately, dissolve the pellet in a minimal amount of sterile, double dist. water, then dilute an aliquot of the probe solution to a convenient stock concentration (e.g., 1–7 ng/ μ l) in the hybridization buffer to be used for the *in situ* experiment (as described in Chapters 2 and 5 of this manual).*

9 The efficiency of the tailing reaction can be checked by comparison with the tailed control-oligonucleotide (vial 8) in hybridization or direct detection. It is recommended to routinely check the tailing efficiency by direct detection (see DIG Nucleic Acid Detection Kit).

10 The tailed oligonucleotide can be analyzed by polyacrylamide-gel electrophoresis and subsequent silver staining in comparison to the untailed oligonucleotide (vial 7). DIG-tailing of oligonucleotides results in a heterogeneous shift to higher molecular weight and is detectable as a smear in polyacrylamide gels. The control oligonucleotide tailed in the standard reaction is completely shifted to the labeled form.

 *It is not recommended to increase the amount of oligonucleotide in the tailing reaction. Larger amounts of oligonucleotide may be labeled by increasing the reaction volume and all components proportionally.*

Stability of labeled probes

DIG-labeled probes can be stored at -15 to -25°C for at least 1 year.

Reagents available from Roche Applied Science for this procedure

Reagent	Description	Available as
DIG Oligonucleotide Tailing Kit, 2nd generation*		▶ (Cat. No. 03 353 583 910) kit for 25 tailing reactions of 100 pmol oligonucleotides
5× Reaction buffer	1 M potassium cacodylate, 125 mM Tris-HCl, (Cat. No. 1 417 231) 1.25 mg/ml bovine serum albumin, pH 6.6 (+25°C)	▶ Vial 1, DIG Oligonucleotide Tailing Kit, 2 nd generation (Cat. No. 03 353 583 910) ▶ Supplied with Terminal Transferase
CoCl₂ solution	25 mM cobalt chloride (CoCl ₂)	▶ Vial 2, DIG Oligonucleotide Tailing Kit, 2 nd generation (Cat. No. 03 353 583 910) ▶ Supplied with Terminal Transferase
DIG-dUTP	1 mM Digoxigenin-11-dUTP (2'-dideoxyuridine-5'- triphosphate, coupled to digoxigenin via an 11-atom spacer arm) in double dist. water	▶ Vial 3, DIG Oligonucleotide Tailing Kit, 2 nd generation (Cat. No. 03 353 583 910) ▶ DIG-dUTP, alkali-labile (Cat. Nos. 11 573 152 910, 11 573 179 910) ▶ DIG-dUTP, alkali-stable (Cat. Nos. 11 093 088 910, 11 558 706 910, 11 570 013 910)
dATP	Lithium salt, 10 mM dATP solution; in Tris buffer, pH 7.5	▶ Vial 4, DIG Oligonucleotide Tailing Kit, 2 nd generation (Cat. No. 03 353 583 910) ▶ dATP [Cat. No. 11 051 440 001 (sold as a 100 mM solution; must be diluted before use)]
Terminal Transferase recombinant from <i>E.coli</i>	400 U/μl Terminal Transferase, in 60 mM K-phosphate (pH 7.2 at 4°C), 150 mM KCl 1 mM 2-Mercaptoethanol 0.5% Triton X-100 50% Glycerol	▶ Vial 5, DIG Oligonucleotide Tailing Kit, 2 nd generation (Cat. No. 03 353 583 910) ▶ Terminal Transferase Cat. No. 03 333 566 001 8.000 U for 20 tailing or 3'-end labeling reactions ▶ Terminal Transferase Cat. No. 03 333 574 001 24.000 U for 60 tailing or 3'-end labeling reactions
Glycogen from mussels	20 mg/ml, aqueous solution	▶ Cat. No. 10 901 393 001

* The labeling of nucleic acids with DIG is covered by EP patents 0 324 474 and 0 371 262 as well as the following US patents 5.344.757, 5.354.657 and 5.702.888 owned by Roche Diagnostics GmbH.

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