






VI. Oligonucleotide 3'-end labeling with DIG-ddUTP or Biotin-ddUTP



Terminal Transferase is used to add a single modified dideoxyuridine-triphosphate (e.g., DIG-ddUTP) to the 3' ends of an oligonucleotide (Schmitz et al., 1990).

 *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-ddUTP (X = DIG or Biotin) in double dist. water.
- 3** Add the following to a microcentrifuge tube on ice:
 -  *The 5x reaction buffer and the Terminal Transferase contain toxic material. Handle with care.*
 - ▶ 4 µl of 5x 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 3'-End Labeling 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-ddUTP (vial 3) or 1 mM Biotin-ddUTP.
 - ▶ 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 4).
 - ▶ 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, in double dist. water) (vial 8).
 - ▶ 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 µl 4 M LiCl and 75 µl 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 × g) for 15 min at 2–8°C.
 - ▶ Discard the supernatant.
 - ▶ Wash the pellet with 50 µl ice-cold 70% (v/v) ethanol.
 - ▶ Centrifuge the tube (at 13,000 × g) for 5 min at 2–8°C.
 - ▶ Discard the supernatant.
 - ▶ Dry the pellet under vacuum.



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- 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 for last 1 year.
 *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/μl) in the hybridization buffer to be used for the *in situ* experiment (as described in Chapters 2 and 5 of this manual).
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- 9** The efficiency of the labeling reaction can be checked by comparison with the labeled control-oligonucleotide (vial 6) in hybridization or direct detection. It is recommended to routinely check the labeling efficiency by direct detection (see page 60).
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- 10** The labeled oligonucleotide can be analyzed by polyacrylamide-gel electrophoresis and subsequent silver staining in comparison to the unlabeled oligonucleotide. DIG labeled oligonucleotides are shifted to higher molecular weight due to the addition of the label. The control oligonucleotide labeled in the standard reaction is completely shifted to the end labeled form.
-  *It is not recommended to increase the amount of oligonucleotide in the labeling reaction. Larger amounts of oligonucleotide can be labeled by increasing the reaction volume and all components proportionally.*
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Reagents available from Roche Applied Science for this procedure

Reagent	Description	Available as
DIG Oligonucleotide 3'-End Labeling Kit, 2nd generation*		▶ (Cat. No. 03 353 575 910) kit for 25 labeling reactions of 100 pmol oligonucleotides
5× Reaction buffer	1 M potassium cacodylate, 125 mM Tris-HCl, 1.25 mg/ml bovine serum albumin, pH 6.6 (+25°C)	▶ Vial 1, DIG Oligonucleotide 3'-End Labeling Kit, 2 nd generation (Cat. No. 03 353 575 910) ▶ Supplied with Terminal Transferase
CoCl₂ solution	25 mM cobalt chloride (CoCl ₂)	▶ Vial 2, DIG Oligonucleotide 3'-End Labeling Kit, 2 nd generation (Cat. No. 03 353 575 910) ▶ Supplied with Terminal Trans- ferase
DIG-ddUTP*	1 mM Digoxigenin-11-ddUTP (2', 3'-dideoxyuridine-5'- triphosphate, coupled to digoxigenin via an 11-atom spacer arm) in double dist. water	▶ Vial 3, DIG Oligonucleotide 3'-End Labeling Kit, 2 nd generation (Cat. No. 03 353 575 910) ▶ DIG-ddUTP (Cat. No. 11 363 905 910)
Biotin-16-ddUTP	1 mM tetralithium salt, solution	▶ Cat. No. 11 427 598 910, 25 nmol (25 µl)
Terminal Transferase re- combinant from <i>E.coli</i>	400 U/µl Terminal Transferase, in 60 mM K-phosphate (pH 7.2 at 4°C), 150 mM KCE 1 mM 2-Mercaptoethanol 0.5% Triton X-100 50% Glycerol	▶ Vial 4, DIG Oligonucleotide 3'-End Labeling Kit, 2 nd generation (Cat. No. 03 353 575 910) ▶ 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

* 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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