For general laboratory use.

Terminal Transferase
from Calf Thymus, recombinant, *E. coli*

Version: 06
Content Version: April 2020

Nucleoside triphosphate: DNA deoxynucleotidylexotransferase

Cat. No. 03 333 566 001	8,000 U 400 U/µl 20 tailing or 3'-end labeling reactions (400 U per reaction)
Cat. No. 03 333 574 001	24,000 U 400 U/µl 60 tailing or 3'-end labeling reactions (400 U per reaction)

Store the product at −15 to −25°C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Catalog Number	Content
1	Terminal Transferase, rec.	Enzyme storage buffer: 60 mM potassium phosphate (pH 7.2 at +4°C), 150 mM KCl, 1 mM 2-mercaptoethanol, 0.5% Triton X-100, 50% glycerol.	03 333 566 001	1 vial, 20 µl
			03 333 574 001	1 vial, 60 µl
2	TdT Reaction buffer, 5x conc.	Contains 1 M potassium cacodylate, 125 mM Tris-HCl, 1.25 mg/ml BSA (pH 6.6 at +25°C).	03 333 566 001	1 vial, 1 ml
			03 333 574 001	
3	$CoCl_2$ solution, 25 mM	For tailing and end-labeling reactions.	03 333 566 001	1 vial, 1 ml
			03 333 574 001	

1.2. Storage and Stability

Storage Conditions (Product)

When stored at −15 to −25°C, the product is stable through the expiry date printed on the label.

Vial / Bottle	Label	Storage
1	Terminal Transferase, rec.	Store at −15 to −25°C.
2	TdT Reaction buffer, 5x conc.	
3	$CoCl_2$ solution, 25 mM	

1.3. Additional Equipment and Reagent required

Standard tailing reaction with nonradioactive nucleotides

- Digoxigenin-11-dUTP, alkali-stable* or
- Digoxigenin-11-dUTP, alkali-labile*
- 0.2 M EDTA, pH 8.0
- Set of Deoxynucleotides* or
- dATP*
- dGTP*
- dCTP*
- dTTP*
- Double-distilled water

Standard tailing reaction with radioactive nucleotides

- 0.2 M EDTA, pH 8.0
- [α-^{32}P]dATP (800 Ci/mmol, approximately 30 TBq/mmol)
- [α-^{32}P]dTTP (800 Ci/mmol, approximately 30 TBq/mmol)
- [α-^{32}P]dGTP (800 Ci/mmol, approximately 30 TBq/mmol)
- [α-^{32}P]dCTP (800 Ci/mmol, approximately 30 TBq/mmol)
- Set of Deoxynucleotides* or
- dATP*
- dGTP*
- dCTP*
- dTTP*
- Double-distilled water

Standard 3′-end labeling reaction with nonradioactive nucleotides

- DIG-11-ddUTP*, 1 mM
- 0.2 M EDTA, pH 8.0
- Double-distilled water

Standard 3′-end labeling reaction with radioactive nucleotides

- [α-^{32}P]ddATP (3,000 Ci/mmol, approximately 110 TBq/mmol)
- 0.2 M EDTA, pH 8.0
- Double-distilled water

1.4. Application

Terminal Transferase is used for:

Tailing with dNTPs

- Addition of homopolymeric tails to DNA fragments.
- Labeling of double- and single-stranded DNA and oligonucleotides with either radioactive or chemically modified nucleotides such as DIG-11-dUTP*.

3′-end labeling with ddNTPs

- Labeling of double- and single-stranded DNA and oligonucleotides with either radioactive or chemically modified dideoxynucleotides such as DIG-11-ddUTP*.

Product Description

Terminal Transferase catalyzes the template independent addition of deoxy- and dideoxynucleoside triphosphates to the 3′-OH ends of double- and single-stranded DNA fragments and oligonucleotides. Terminal Transferase incorporates digoxigenin-, biotin-, and fluorochrome-labeled deoxy- and dideoxynucleoside triphosphates* as well as radioactively labeled deoxy- and dideoxynucleoside triphosphates. The supplied 5x-concentrated reaction buffer allows the optimal tailing of all types of double-stranded DNA ends: blunt ended, with 3′ overhang or with 5′ overhang. The highest incorporation rates are obtained with 3′ overhangs.

2. How to Use this Product

2.1. Before you Begin

Safety Information

Precautions

The reaction buffer for Terminal Transferase contains potassium cacodylate.
- Toxic by inhalation and if swallowed.
- After contact with skin, wash immediately with sufficient amounts of water.
- In case of an accident or if you feel ill, seek medical advice immediately.

Working Solution

Standard tailing reaction with radioactive nucleotides

Solution	Preparation
$CoCl_2$ working solution	Add in a sterile vial, 10 µl double-distilled water and 15 µl of 25 mM $CoCl_2$ solution (Vial 3): Final concentration: 15 mM
dATP and dTTP labeling mix	Mix 1 volume of a 2.5 mM dATP or dTTP solution with 15 volumes of double-distilled water and 4 volumes of [α-^{32}P]dATP or [α-^{32}P]dTTP (800 Ci/mmol, approximately 30 TBq/mmol).
dGTP and dCTP labeling mix	Mix 1 volume of a 2 mM dGTP or dCTP solution with 15 volumes of double-distilled water and 4 volumes of [α-^{32}P]dGTP or [α-^{32}P]dCTP (800 Ci/mmol, approximately 30 TBq/mmol).

2.2. Protocols

Standard tailing reaction with nonradioactive nucleotides

1. Add the following to a reaction vial:

Reagent	Volume [µl]	Final conc.
TdT Reaction buffer, 5x conc.	4	1x
Template	X	approximately 100 pmol 3′ ends
DIG-11-dUTP solution (1 mM)	1	0.05 mM
$CoCl_2$, 25 mM	4	5 mM
DIG-11-dUTP/dATP tailing (dATP, 10 mM)	1	0.5 mM
DIG-11-dUTP/dTTP tailing (dTTP, 10 mM)	1	0.5 mM
DIG-11-dUTP/dGTP tailing (dGTP, 10 mM)	1	0.5 mM
DIG-11-dUTP/dCTP tailing (dCTP, 10 mM)	1	0.5 mM
Terminal Transferase, rec.	1	400 U/reaction
Double-distilled water	add up to 20 µl	
Total volume	**20 µl**	

– Mix and centrifuge briefly.
– Incubate for 15 minutes at +37°C, then place on ice.

2. Stop the reaction by adding 2 µl 0.2 M EDTA, pH 8.0.

Tail length

The tail length reflects the number of incorporated DIG molecules and as such the labeling efficiency. It is dependent on the type and concentration of deoxynucleoside triphosphates and the ratio of DIG-dUTP to unlabeled nucleotides. The **Standard tailing reaction with nonradioactive nucleotides** gives the following results when using a DIG-dUTP/dNTP labeling mixture of 1/10.

Tailing with	DIG-dUTP/dATP	DIG-dUTP/dTTP	DIG-dUTP/dGTP	DIG-dUTP/dCTP
Average tail length	50	10	15	25
Range of tail length	10 – 100	1 – 20	10 – 5	10 – 40
Amount of DIG-11-dUTP molecules/tail	5	1	1.5	2.5

Standard tailing reaction with radioactive nucleotides

See section, **Working Solution** *for additional information on preparing solutions.*

1 Add the following to a reaction vial:

Reagent	Tailing [µl]				Final conc.
	A	T	G	C	
TdT Reaction buffer, 5x conc.	4	4	4	4	1x
Template	X	X	X	X	approximately 1 pmol 3′ ends
$CoCl_2$ working solution (15 mM)	2	2	1	1	1.5 mM (2 µl), 0.75 mM (1 µl)
dATP labeling mix	1	–	–	–	6.25 µM
dTTP labeling mix	–	1	–	–	6.25 µM
dGTP labeling mix	–	–	1	–	5 µM
dCTP labeling mix	–	–	–	1	5 µM
Terminal Transferase, rec.	1	1	1	1	400 U/reaction
Double-distilled water	add up to 20 µl	add up to 20 µl	add up to 20 µl	add up to 20 µl	
Final volume	**20 µl**	**20 µl**	**20 µl**	**20 µl**	

– Mix and centrifuge briefly.
– Incubate for 15 minutes at +37°C, then place on ice.

2 Stop the reaction by adding 2 µl 0.2 M EDTA, pH 8.0.

Tail length

Tailing with	[α-^{32}P]dATP/dATP	[α-^{32}P]dTTP/dTTP	[α-^{32}P]dGTP/dGTP	[α-^{32}P]dCTP/dCTP
Range of tail length	75 – 125	75 – 125	15 – 30	15 – 30

Standard 3′-end labeling reaction with nonradioactive nucleotides

1. Add the following to a reaction vial:

Reagent	Volume [µl]	Final conc.
TdT Reaction buffer, 5x conc.	4	1x
Template	X	approximately 100 pmol 3′ ends
$CoCl_2$, 25 mM	4	5 mM
DIG-11-dUTP solution (1 mM)	1	0.05 mM
Terminal Transferase, rec.	1	400 U/reaction
Double-distilled water	add up to 20 µl	
Final volume	**20 µl**	

– Mix and centrifuge briefly.
– Incubate for 15 minutes at +37°C, then place on ice.

2. Stop the reaction by adding 2 µl 0.2 M EDTA, pH 8.0.

Standard 3′-end labeling reaction with radioactive nucleotides

1. Add the following to a reaction vial:

Reagent	Volume [µl]	Final conc.
TdT Reaction buffer, 5x conc.	10	1x
Template	X	approximately 10 pmol 3′ ends
$CoCl_2$, 25 mM	5	2.5 mM
[α-^{32}P]ddATP, 3,000 Ci/mmol (approximately 110 TBq/mmol)	5	–
Terminal Transferase, rec.	1	400 U/reaction
Double-distilled water	add up to 50 µl	
Final volume	**50 µl**	

– Mix and centrifuge briefly.
– Incubate for 60 minutes at +37°C, then place on ice.

2. Stop the reaction by adding 5 µl 0.2 M EDTA, pH 8.0.

i The incorporation rate is determined by comparing incorporated radioactivity to total input radioactivity.

Incorporation efficiency

By using the standard protocol, an incorporation rate of at least 30% is obtained.

Calculation of amount of pmol 3′ ends

- pmol of 3′ends of a dsDNA molecule

$$= \frac{2 \times 10^6 \times \mu g \text{ (of dsDNA)}}{\text{MW (in Da)}} = \frac{2 \times 10^6 \times \mu g \text{ (of dsDNA)}}{N_{bp} \times 660 \text{ (Da)}}$$

e.g. pmol of 3′ends of 1 µg of a 100 bp dsDNA fragment.

$$= \frac{2 \times 10^6 \times 1}{100 \times 660} = 30.3$$

- pmol of 3′ends of a ssDNA molecule

$$= \frac{2 \times 10^6 \times \mu g \text{ (of ssDNA)}}{\text{MW (in Da)}} = \frac{1 \times 10^6 \times \mu g \text{ (of ssDNA)}}{N_b \times 330 \text{ (Da)}}$$

e.g. pmol of 3′ends of 1 µg of a 250 b ssDNA fragment

$$= \frac{1 \times 10^6 \times 1}{250 \times 330} = 12.12$$

- pmol of 3′ends generated by restriction endonuclease cleavage:

  circular DNA:
  2 × (pmol of DNA) × (number of sites)

  linear DNA:
  [2 × (pmol of DNA) × (number of sites)] +
  [2× (pmol of DNA)]

N_{bp} = number of basepairs (dsDNA) and
N_b = number of bases (ssDNA)

Fig. 1: Calculation of amount of pmol 3’ ends.

2.3. Parameters

Unit Assay

Unit assay conditions: 200 mM potassium cacodylate,1 mM $CoCl_2$, 1 mM dTTP, 0.1 OD $d(pT)_6$, 6.25 pmol [^{3}H]dTTP in a 120 µl reaction volume.

Unit Definition

One unit is the enzyme activity that incorporates 1 nMol dTMP into acid-insoluble products within 30 minutes at +37°C under assay conditions using $d(pT)_6$ as primer.

Volume Activity

400 U/µl
400 U (1 µl) are needed for one tailing or one 3′-end labeling reaction.

3. Additional Information on this Product

3.1. Quality Control

For lot-specific certificates of analysis, see section, **Contact and Support**.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.
Editorial changes.

4.3. Ordering Information

Product	Pack Size	Cat. No.
Reagents, kits		
Digoxigenin-11-dUTP, alkali-labile	25 nmol, 25 µl, 1 mM	11 573 152 910
	125 nmol, 125 µl, 1 mM	11 573 179 910
Deoxynucleoside Triphosphate Set	4 x 250 µl, 4 x 25 µmol, 100 mM, 6,250 reactions at 20 µl final volume.	11 969 064 001
	4 x 1,250 µl, 4 x 125 µmo, 100 mM, 37,500 reactions at 20 µl final volume.	03 622 614 001
Digoxigenin-11-dUTP, alkali-stable	25 nmol, 25 µl, 1 mM	11 093 088 910
	125 nmol, 125 µl, 1 mM	11 558 706 910
	5 x 125 nmol, 5x 125 µl, 1 mM	11 570 013 910
Digoxigenin-11-ddUTP	25 nmol, 25 µl, 1 mM	11 363 905 910
Biotin-16-dUTP	50 nmol, 50 µl, 1 mM	11 093 070 910
Biotin-16-ddUTP	25 nmol, 25 µl , 1 mM	11 427 598 910
Fluorescein-12-dUTP	25 nmol, 25 µl, 1 mM	11 373 242 910
dATP	250 µl, 25 µmol, 100 mM, 6,250 standard PCR assays of 20 µl each.	11 934 511 001
	1,250 µl, 125 µmol, 100 mM, 31,250 standard PCR assays of 20 µl each.	11 969 013 001
	4 x 1,250 µl, 4 x 125 µmol, 100 mM	03 732 681 001
dCTP	250 µl, 25 µmol, 100 mM, 6,250 standard PCR assays of 20 µl each.	11 934 520 001
	1,250 µl, 125 µmol, 100 mM, 31,250 standard PCR assays of 20 µl each.	11 969 021 001
	4 x 1,250 µl, 4 x 125 µmo, 100 mM	03 732 690 001
dGTP	250 µl, 25 µmol, 100 mM, 6,250 standard PCR assays of 20 µl each.	11 934 538 001
	1,250 µl, 125 µmol, 100 mM, 31,250 standard PCR assays of 20 µl each.	11 969 030 001
	4 x 1,250 µl, 4 x 125 µmo, 100 mM	03 732 703 001
dTTP	250 µl, 25 µmol, 100 mM, 6,250 standard PCR assays of 20 µl each.	11 934 546 001
	1,250 µl, 125 µmol, 100 mM, 31,250 standard PCR assays of 20 µl each.	11 969 048 001

4.4. Trademarks

All product names and trademarks are the property of their respective owners.

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products.

4.6. Regulatory Disclaimer

For general laboratory use.

4.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.8. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site**.

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

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