For life science research only.
Not for use in diagnostic procedures.

SP6/T7 Transcription Kit

Version: 20
Content Version: November 2021

For *in vitro* transcription of DNA.

Cat. No. 10 999 644 001 1 kit
2 x 20 transcription assays

Store the kit at −15 to −25°C.

1. General Information

1.1. Contents

Vial / bottle	Label	Function / description	Content
1	SP6/T7 Transcription Kit, pSPT 18 DNA	▪ Solution, 0.25 mg/ml ▪ Cloning/transcription vector; subclones are transcribed into RNA probes by T7 or SP6 RNA Polymerase.	1 vial, 40 µl
2	SP6/T7 Transcription Kit, pSPT 19 DNA	▪ Solution, 0.25 mg/ml ▪ Cloning/transcription vector; subclones are transcribed into RNA probes by T7 or SP6 RNA Polymerase.	1 vial, 40 µl
3	SP6/T7 Transcription Kit, Control DNA	▪ Control DNA mixture ▪ 5 µg each pSPT 18- and pSPT 19-neo-DNA, cleaved with Eco RI.	1 vial, 20 µl
4	SP6/T7 Transcription Kit, ATP	10 mM ATP in Tris buffer.	1 vial, 40 µl
5	SP6/T7 Transcription Kit, CTP	10 mM CTP in Tris buffer.	1 vial, 40 µl
6	SP6/T7 Transcription Kit, GTP	10 mM GTP in Tris buffer.	1 vial, 40 µl
7	SP6/T7 Transcription Kit, UTP	10 mM UTP in Tris buffer.	1 vial, 40 µl
8	SP6/T7 Transcription Kit, Buffer, 10x conc.	Transcription buffer for RNA labeling reaction.	1 vial, 100 µl
9	SP6/T7 Transcription Kit, DNase I, RNase-free	▪ Solution, 10 U/µl in buffer with 50% glycerol. ▪ Degrades DNA template after the labeling reaction.	1 vial, 20 µl
10	SP6/T7 Transcription Kit, RNase Inhibitor	▪ Solution, 20 U/µl in buffer with 50% glycerol. ▪ Prevents the degradation of RNA during the labeling reaction.	1 vial, 20 µl
11	SP6/T7 Transcription Kit, SP6 RNA Polymerase	▪ Solution, 10 U/µl in buffer with 50% glycerol. ▪ Synthesizes RNA from a DNA template.	1 vial, 20 µl
12	SP6/T7 Transcription Kit, T7 RNA Polymerase	▪ Solution, 10 U/µl in buffer with 50% glycerol. ▪ Synthesizes RNA from a DNA template.	1 vial, 20 µl

1.2. Storage and Stability

Storage Conditions (Product)

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

Vial / bottle	Label	Storage
1	pSPT 18 DNA	Store at −15 to −25°C. ⚠ ***Avoid repeated freezing and thawing.***
2	pSPT 19 DNA	
3	Control DNA	
4	ATP	
5	CTP	
6	GTP	
7	UTP	
8	Buffer, 10x conc.	
9	DNase I, RNase-free	
10	RNase Inhibitor	
11	SP6 RNA Polymerase	
12	T7 RNA Polymerase	

1.3. Additional Equipment and Reagent required

For the radioactive assay

- Water bath or heating block
- Template DNA
- Labeled nucleotide [α-^{32}P] CTP (400 Ci/mmol)
- Water, PCR Grade*
- 0.2 M EDTA, pH 8.0

For the nonradioactive assay

- Water bath or heating block
- Template DNA
- Water, PCR Grade*
- Digoxigenin-11-UTP*, or
- Biotin-16-UTP*, or
- Fluorescein-12-UTP*
- 0.2 M EDTA, pH 8.0

For cold assay

- Water bath or heating block
- Template DNA
- Water, PCR Grade*
- 0.2 M EDTA, pH 8.0

For removal of unincorporated nucleotides

- Quick Spin Columns for radiolabeled DNA purification Sephadex G-50*

For determination of labeling efficiency

- Trichloroacetic
- DIG RNA Labeling Kit (SP6/T7)*

1.4. Application

Labeled transcripts lend themselves to all DNA and RNA hybridization techniques and are also used for genomic sequencing and S1 nuclease studies.

- Large amounts of highly pure RNA can be synthesized using the SP6/T7 system. These transcripts are used for studies on RNA-processing systems.
- Synthesized RNA can be translated *in vitro* or *in vivo* after injection into oocytes. The transcription of defined mRNA can be inhibited by the introduction of anti-sense-RNA.
- The efficiency of *in vivo* translation of synthesized mRNA can be increased significantly by the introduction of a cap structure.

Transcripts produced with SP6 or T7 RNA polymerases exhibit the following:

- High levels of specific labeling.
- The length of transcripts can be exactly controlled by linearization of the DNA template, enabling the production of long labeled fragments >4 kb.
- The single-stranded nature of the transcripts prevents renaturation, as occurs with double-stranded DNA probes.
- Single-stranded probes hybridize specifically onto their complementary strand, and are therefore useful for screening M13 clones of specific orientation.
- The properties of DNA/RNA hybrids allow hybridization under more stringent conditions than with labeled DNA probes.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

DNA inserted into the transcription vectors pSPT 18 or pSPT 19.

The template DNA must be linearized with a suitable restriction enzyme before the transcription reaction to obtain transcripts of a defined length. Using intact plasmid DNA as template for transcription will result in heterogeneous transcripts of multiple plasmid lengths. The DNA template may be removed by digestion with DNase I, RNase-free after the reaction.

Control Reactions

Transcript length of controls

Transcripts of 1,028 or 1,035 bases, respectively are obtained with SP6 or T7 RNA Polymerase from the Control DNA (Vial 3) in the standard assay. Under standard assay conditions >50% of the label is incorporated in 20 minutes into RNA transcripts.

General Considerations

Number of labeling reactions

2 × 20 standard transcription assays may be performed with the kit. However, twice as many reactions may be performed with our Control DNA (Vial 3) or with highly purified DNA using 5 U of the respective RNA polymerase per assay. This may result in approximately 3 to 5% lower incorporation. Increasing the temperature to +40°C will result in a slight increase in incorporation. RNA may be synthesized in the presence of labeled or unlabeled ribonucleoside triphosphates.

Kinetics of the labeling reaction

The yield and extent of specific labeling and the kinetics of the labeling reaction depend on the ratio of DNA to labeled ribonucleoside triphosphate. The standard assay uses 0.5 µg of the corresponding template contained in the Control DNA (solution 3; 0.5 µg pSPT 18-neo DNA when using SP6 RNA polymerase and 0.5 µg pSPT 19-neo DNA when using T7 RNA polymerase, respectively) and 50 µCi α^{32}P-CTP, 400 Ci/mmol. Ribonucleoside triphosphates with specific activities of 3,000 Ci/mmol must be supplemented with the analogous cold ribonucleoside triphosphate to obtain full length transcripts. For example, when using α^{32}P-CTP, 3,000 Ci/mmol, supplement the standard assay with 1 µl CTP (Vial 5, diluted 1 + 49 with autoclaved double-distilled water).

Working Solution

Solution	Preparation/Composition	For use in...
ATP, GTP, UTP mixture	Mix solutions from Vials 4, 6, and 7 in a 1:1:1 ratio.	Standard labeling assay.
ATP, GTP, CTP mixture	Mix solutions from Vials 4, 5, and 6 in a 1:1:1 ratio.	Transcription assay with DIG-11-UTP.
UTP/DIG-11-UTP mixture	Mix 6 mM DIG-11-UTP with UTP (Vial 7) in a 1:1 ratio.	Transcription assay with DIG-11-UTP.
ATP, GTP, CTP, UTP mixture	Mix solutions from the Vials 4, 5, 6, and 7 in a 1:1:1:1 ratio.	Cold transcription

2.2. Protocols

Standard labeling assay

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

1 Pipette the following components into a microfuge tube on ice.

1 µl RNase Inhibitor may be added to the reaction to ensure the preservation of the full-length single-stranded RNA molecules, if necessary.

Reagent	Volume [µl]	Control DNA (Vial 3) [µl]
0.5 µg Template DNA	variable	2
Nucleotides ATP, GTP, UTP	3	3
Labeled [α^{32}P] CTP, 400 Ci/mmol, aqueous solution	5	5
Buffer, 10x conc. (Vial 8)	2	2
SP6 or T7 RNA Polymerase (Vials 11 or 12)	1	1
Water, PCR Grade*	add up to a final volume of 18	7
Final Volume	**20**	**20**

– Mix and centrifuge briefly.
– Incubate for 20 minutes at +37°C.

2 Optional: Add 2 µl DNase I, RNase-free (Vial 9) to remove the DNA template.
– Incubate for 15 minutes at +37°C.

Essential step for RNase-protection experiments.

3 Stop the reaction by adding 2 µl 0.2 M EDTA, pH 8.0 and/or heat to +65°C for 10 minutes.

The RNA transcripts can be checked for length and integrity by native or denaturing gel electrophoresis.

4 Remove nonincorporated ribonucleoside triphosphates using Quick Spin Columns, Sepahdex G-50 or repeated ethanol precipitations.

Labeling efficiency

The level of labeling may be determined by measuring the radioactivity incorporated into an aliquot of the assay in comparison to total input radioactivity. The kinetics of incorporation may be monitored by removal of aliquots at varying time points and trichloroacetic acid precipitation.
Elevating the ratio of radioactive ribonucleoside triphosphate to DNA substrate, allows higher specific labeling with retarded kinetics of reaction. Thus, using 0.5 µg of the corresponding template contained in the Control DNA and 200 µCi, >40% of the input radioactivity are incorporated in 45 minutes.

Transcription assay with DIG-11-UTP

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

1 Pipette the following components into a microfuge tube on ice.

Biotin- or Fluorescein-labeled UTP can also be used.*

Reagent	Volume [µl]	Control DNA (Vial 3) [µl]
1 µg Template DNA	variable	4
Nucleotides ATP, GTP, CTP	6	6
UTP/DIG-11-UTP* mixture	2	2
Buffer, 10x conc. (Vial 8)	2	2
SP6 or T7 RNA Polymerase (Vials 11 or 12)	2	2
RNase Inhibitor (Vial 10)	1	1
Water, PCR Grade*	add up to a final volume of 18 µl	3
Final Volume	**20**	**20**

– Mix and centrifuge briefly.
– Incubate for 2 hours at +37°C.

Longer incubations do not increase the yield of labeled RNA.

2 Optional: Add 2 µl DNase I, RNase-free (Vial 9) to remove the DNA template.
– Incubate for 15 minutes at +37°C.

Essential step for RNase-protection experiments.

3 Stop the reaction by adding 2 µl 0.2 M EDTA, pH 8.0 and/or heat to +65°C for 10 minutes

The RNA transcripts can be checked for length and integrity by native or denaturing gel electrophoresis.

Labeling efficiency

The labeling efficiency is determined by semi-quantitative determination. The protocol is available in several DIG Labeling kits, such as the DIG RNA Labeling Kit (SP6/T7)*.

Cold assay

See section, **Working Solution** *for information on preparing solutions.*
For this assay, all four ribonucleoside triphosphates are used unlabeled. The substrate levels are not limiting and the synthesis continues longer producing maximum amounts of RNA using the standard 1 hour assay. Up to 15 µg of RNA transcripts may be obtained from 1 µg of the corresponding template contained in the Control DNA.

RNase Inhibitor may be added to the reaction to ensure the preservation of the full-length single-stranded RNA molecules, if necessary.

1 Pipette the following components into a microfuge tube on ice.

Reagent	Volume [µl]	Control DNA (Vial 3) [µl]
0.5 µg Template DNA	variable	4
Nucleotides ATP, GTP, CTP, UTP	8	8
Buffer, 10x conc. (Vial 8)	2	2
SP6 or T7 RNA Polymerase (Vials 11 or 12)	2	2
Water, PCR Grade*	add up to a final volume of 18 µl	4
Final Volume	**20**	**20**

– Mix and centrifuge briefly.
– Incubate for 1 hour at +37°C.

2. Optional: Add 2 µl DNase I, RNase-free (Vial 9) to remove the DNA template.
   – Incubate for 15 minutes at +37°C.

   Essential step for RNase-protection experiments.

3. Stop the reaction by adding 2 µl 0.2 M EDTA, pH 8.0 and/or heat to +65°C for 10 minutes.

   The RNA transcripts can be checked for length and integrity by native or denaturing gel electrophoresis.

4. Optional: Transcripts can be purified from enzyme activity by phenol extraction.

2.3. Parameters

Activator

Spermidine stimulates SP6 and T7 RNA Polymerase activity.

Cofactors

SP6 and T7 RNA Polymerases require a DNA template and Mg^{2+} for the synthesis of RNA.

Specificity

Promoter specificity

SP6 and T7 RNA polymerases are extremely promoter-specific and only transcribes bacteriophage SP6/T7 DNA or DNA cloned downstream of a SP6/T7 promoter.

3. Additional Information on this Product

3.1. Test Principle

Transcription and labeling

During RNA labeling by *in vitro* transcription, DNA to be transcribed is cloned into the polylinker site of appropriate transcription vectors, such as pSPT 18 or 19, which contain promoters for SP6 and T7 RNA polymerases. These two vectors differ only in the orientation of their polylinker regions.

- The promoters for SP6 and T7 RNA polymerases are located on either side of the polylinker. SP6 and T7 RNA polymerases specifically transcribe DNA sequences downstream of the SP6 or T7 promoters, respectively.
- Cloned inserts within the polylinker region are transcribed from either promoter. The first DNA strand may be transcribed with SP6 RNA polymerase and the opposite strand using T7 RNA polymerase.
- It is also possible to transcribe the first and opposite strands by inserting the same DNA into both pSPT 18 and pSPT 19 in opposite orientations and transcribing with only one of the RNA polymerases.
- SP6 and T7 RNA polymerase use the cloned DNA as template and synthesize complementary RNA in the presence of Mg^{2+} and ribonucleoside triphosphates.
- Specifically labeled transcripts are obtained when using radioactively labeled, for example, ^{32}P, ^{3}H, ^{35}S or nonradioactively labeled, for example, digoxigenin or biotin ribonucleotide triphosphates.

Cloning into pSPT 18/19

The pSPT 18/19 plasmids are pUC derivatives, containing the pBR322 origin and the ampR gene, but no *lac* operon (Fig. 1). Therefore, the presence of these plasmids cannot be detected by blue/white selection methods.

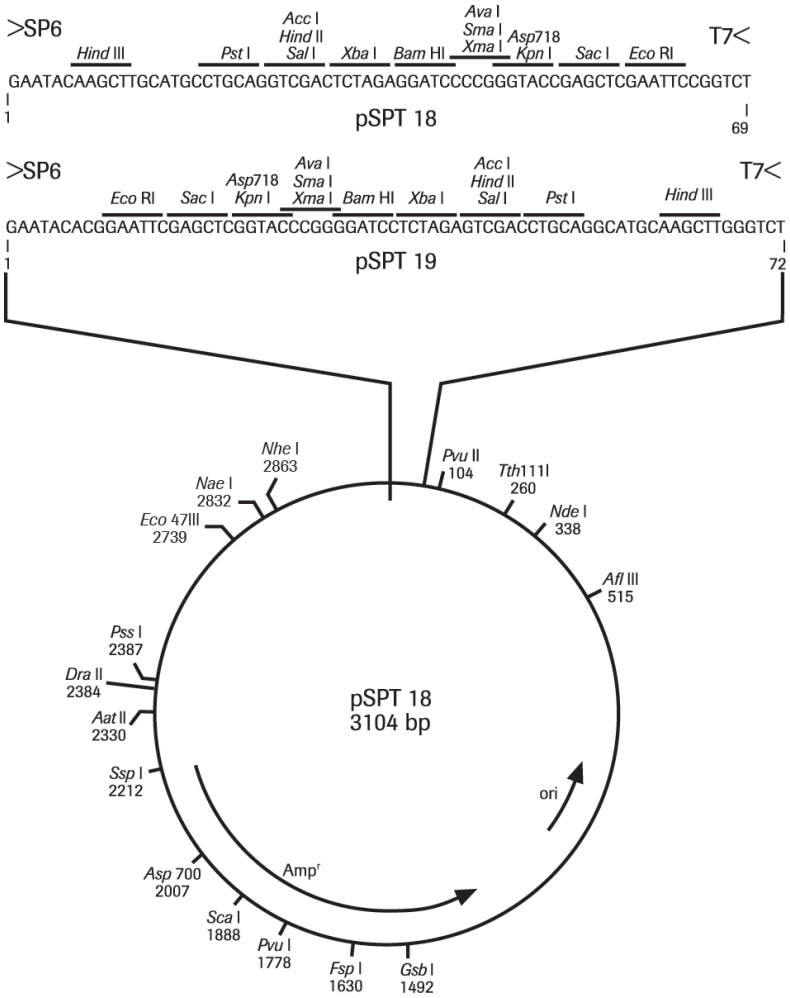

Fig. 1: Transcription vectors pSPT 18 and pSPT 19 plasmid map.

3.2. 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		
Water, PCR Grade	25 ml, 25 x 1 ml	03 315 932 001
	25 ml, 1 x 25 ml	03 315 959 001
	100 ml, 4 x 25 ml	03 315 843 001
Digoxigenin-11-UTP	250 nmol, 25 µl, 10 mM	11 209 256 910
	200 nmol, 57 µl, 3.5 mM	03 359 247 910
Biotin-16-UTP	250 nmol, 25 µl, 10 mM	11 388 908 910
DIG RNA Labeling Kit (SP6/T7)	1 kit, 2 x 10 labeling reactions	11 175 025 910
Fluorescein-12-UTP	250 nmol, 25 µl, 10 mM	11 427 857 910
Quick Spin Columns for radiolabeled DNA purification	20 columns	11 273 965 001
	50 columns	11 273 973 001

4.4. Trademarks

4.5. License Disclaimer

For patent license limitations for individual products please refer to:
List of biochemical reagent products and select the corresponding product catalog.

4.6. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

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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