Genlantis

A division of Gene Therapy Systems, Inc.

TurboScript™ T7 Transcription Kit

PRODUCT SUMMARY

Cat. No: T510003

Description: The TurboScript™ T7 Transcription Kit utilizes a novel technology to allow rapid synthesis of 10 to 50 times the amount of RNA produced by conventional *in vitro* transcription reactions. The secret behind the high yield is that each DNA template is copied hundreds of times. The TurboScript™ T7 Transcription Kit is ideal for preparing double-stranded RNA for use with the Dicer siRNA Construction Kit. Each TurboScript™ T7 Transcription Kit provides sufficient reagents for performing 20 transcription reactions.

Please note: The TurboScript™ T7 Transcription Kit is intended FOR RESEARCH USE ONLY

Components:

Description	Quantity
T7 Enzyme Mix	1 tube (40 µl)
T7 Reaction Buffer	1 tube (40 µl)
NTP Mix	1 tube (160 µl)
DNase I	1 tube (20 µl)
2X Gel Loading Buffer	1 tube (700 µl)
Nuclease-free Water	1 tube (1ml)
LiCl Precipitation Solution	1 tube (700 µl)
GFP Control Plasmid	1 tube (10 µl @ 1µg/µl)
5' Control Primer	1 tube (40 µl @ 1 µg/µl)
3' Control Primer	1 tube (40 µl @ 1 µg/µl)

Shipping and Storage: The TurboScript™ T7 Transcription Kit is shipped frozen. For maximum stability and long-term use, immediately store at –20°C upon receipt. All components are stable for six months when stored properly.

METHODS AND PROCEDURES

1. Generation of RNA

1.1. Thaw the frozen reagents. Place the T7 Enzyme Mix on ice; it is formulated in glycerol and does not freeze at -20°C. Vortex the T7 Reaction Buffer and the NTP Mix until they are completely in solution. Once thawed, store the NTP Mix on ice, and **keep the T7 Reaction Buffer at room temperature while assembling the reaction.**

IMPORTANT: *All reagents should be microfuged briefly before opening to prevent loss and contamination of material that may be present around the rim of the tube.*

1.2. Assemble the transcription reaction **at room temp**. The following amounts are for a single 20 µl reaction. Reactions may be scaled up or down if desired.

Add to 20 µl	Nuclease-free Water
8 µl	NTP mix
2 µl	T7 Reaction Buffer
1 µg	DNA template
2 µl	T7 Enzyme Mix

IMPORTANT: *The spermidine in the T7 Reaction Buffer can co-precipitate the template DNA if the reaction is assembled on ice. Add the T7 Reaction Buffer after the water and the NTP Mix are already in the tube.*

1.3. Gently flick the tube or pipette the mixture up and down gently then microfuge the tube briefly so that the reaction mixture is at the bottom of the tube.

1.4. Incubate at 37°C for 2-4 hours.

TIP *The first time a new template is transcribed, the recommended incubation time is 2–4 hours. To determine the optimum incubation time for maximum yield with a given template, a time-course experiment can be done. To do this, set up a TurboScript™ T7 Transcription reaction, and remove aliquots of the reaction at various intervals (for example after 1 hour, 2 hours, 4 hours, 6 hours, and overnight incubations).*

1.5. Add 1 µl DNase I to each 20 µl T7 Reaction. Mix well and incubate for 15 min at 37°C.

NOTE: *The DNase I treatment removes the template DNA.*

1.6. Check the RNA on a 1% agarose gel (TAE) by using the 2X Gel Loading Buffer.

NOTE: *dsRNA will migrate like DNA i.e. a 500 bp dsRNA will migrate at the same rate as a 500 bp band in a DNA ladder. ssRNA will migrate much faster than a dsDNA of the equivalent size. You may see faint slower-migrating bands above the full-length transcript on non-denaturing gels. These may be the result of secondary structures within the transcript and should be ignored.*

2. Recovery of dsRNA

Double-stranded RNA can be directly use for the Recombinant Dicer Enzyme Kit without purification. However, dsRNA purified using the following procedure can give slightly better results.

2.1. Precipitate the RNA by adding 30 µl Nuclease-free Water and 30 µl LiCl Precipitation Solution to the mixture from Step 1.5.

2.2. Mix thoroughly. Chill for ≥ 30 min at –20°C.

2.3. Centrifuge at 4°C for 15 minutes at maximum speed to pellet the RNA.

2.4. Carefully remove the supernatant. Wash the pellet once with ~1 ml 70% ethanol and centrifuge again to maximize removal of unincorporated nucleotides.

2.5. Carefully remove the 70% ethanol, and resuspend the RNA in Nuclease-free Water or TE Buffer. Determine the RNA concentration and store at –20°C or –70°C.

IMPORTANT *Lithium chloride precipitation may not efficiently precipitate RNAs smaller than 300 nucleotides. Also, the concentration of RNA should be at least 0.1 µg/µl to assure efficient precipitation. To precipitate from TurboScript™ reactions that are thought to have very low yields of RNA, do not dilute the transcription reaction with water prior to adding the LiCl Precipitation Solution.*

3. Quantitation of dsRNA

3.1. Quantitation by UV light absorbance
Reading the A_{260} of a diluted aliquot of the reaction is clearly the simplest way to determine yield, but any unincorporated nucleotides and/or template DNA in the mixture will contribute to the reading. We recommend to do a 1:500 dilution of one aliquot of a TurboScript™ reaction.

For single-stranded RNA, 1 A_{260} unit corresponds to 40 µg/ml, so the RNA yield can be calculated as follows:
A_{260} X dilution factor X 40 = µg/ml RNA.

3.2. Assessing dsRNA yield with RiboGreen®
If you have a fluorometer, or a fluorescence microplate reader, Molecular Probes' RiboGreen® fluorescence-based assay for RNA quantitation is a convenient and sensitive way to measure RNA concentration. Follow the manufacturer's instructions for using RiboGreen®.

3.3. Quantitation by ethidium bromide fluorescence
The intensity of ethidium bromide staining of dsRNA in an agarose gel can be used to get a rough estimation of the RNA yield.

3.4. Ethidium bromide spot assay
If unincorporated nucleotides have been removed, an ethidium bromide spot assay can be used to quantitate RNA concentration. Make a standard curve with several 2 fold dilutions of an RNA solution of known concentration. Start at about 80 ng/µl, and go down to about 1.25 ng/µl. Make a few dilutions of the unknown RNA, and add ethidium bromide to 1 ng/µl to each dilution of both RNAs. Spot 2 µl of the control RNA samples and the unknown RNA dilutions onto plastic wrap placed on a UV transilluminator. Compare the fluorescence of the RNAs to estimate the concentration of the sample RNA. Make sure that the sample dilutions are in the linear range of ethidium bromide fluorescence. This assay will detect as little as 5 ng of RNA with an error of about 2 fold.

3.5. Denaturing gel electrophoresis
If unincorporated nucleotides have not been removed from the reaction, an aliquot of the TurboScript™ reaction should be run on a denaturing agarose or acrylamide gel alongside an aliquot of RNA of known concentration. Stain the samples with ethidium bromide, and simply compare the intensity of the unknown sample to that of the known RNA sample to estimate its concentration.

TROUBLESHOOTING GUIDE

Problem	Possible Causes	Recommended Solutions
Neither my template nor the control reaction works in generating dsRNA.	Expired or defective kit component.	Double check that you have followed the procedure accurately, and consider trying the control reaction a second time. If the kit control still doesn't work, it is an indication that something else may be wrong with the kit. Call Genlantis Technical Support for further troubleshooting.
The control reaction works, but my template gives low dsRNA yield.	Wrong amount of DNA template or poor DNA quality.	Check the amount and quality of template. Also, check an aliquot of the template DNA on an agarose gel to make sure it is intact and that it is the expected size.
	PCR products were of poor quality.	Use a different DNA polymerase if possible and/or extend reaction time.
	DNA template has high G/C content	Optimize transcription reaction condition by doubling the amount of GTP and CTP, performing the reaction at 15°C, and adding 0.5-1% DMSO.

RELATED PRODUCTS

Product	Cat. No.
For efficient generation of siRNA	
Dicer siRNA Construction Kit	
5 genes / 50 transfections	T510001
Recombinant Human Dicer Enzyme Kit	
50 Units	T510002
Recombinant Human Dicer Enzyme Kit	
4 x 50 Units	T510008
RNA Purification Column 1	
20 Columns	T510004
RNA Purification Column 2	
20 Columns	T510005
For efficient and functional siRNA transfection	
GeneSilencer® siRNA Transfection Reagent	
200 reactions (0.75 ml)	T500750
GeneSilencer® siRNA Transfection Reagent	
5 x 200 reactions (5 x 0.75 ml)	T505750
For efficient transfection of DNA into diverse cell lines	
GenePORTER® 2 Transfection Reagent	T202007
75 reactions (0.75 ml)	
GenePORTER® 2 Transfection Reagent	T202015
150 reactions (1.5 ml)	
GenePORTER® 2 Transfection Reagent	T202075
750 reactions (5 x 1.5 ml)	
For 3-minute transformation into E. coli	
TurboCells® Chemically Competent *E. coli*	
20 x 50 µl	C300020
TurboCells® F′ Chemically Competent *E. coli*	
20 x 50 µl	C301020

Please contact us for our latest catalogue of life science research products.

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