TranscriptAid™ T7 High Yield Transcription Kit

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COMPONENTS OF THE KIT

TranscriptAid™ T7 High Yield Transcription Kit	#K0441 **for 50 reactions**
TranscriptAid™ Enzyme Mix	100 µl
5X TranscriptAid™ Reaction Buffer	200 µl
DNase I, RNase-free, 1u/µl	100 µl
ATP Solution*, 100 mM	100 µl
CTP Solution*, 100 mM	100 µl
GTP Solution*, 100 mM	100 µl
UTP Solution*, 100 mM	100 µl
Control template (0.5 µg/µl)	10 µl
3M Sodium Acetate Solution, pH 5.2	1 ml
DEPC-treated Water	1 ml
2X RNA Loading Dye Solution	1 ml
RiboRuler™ RNA Ladder, High Range, ready-to-use	40 µl
0.5 M EDTA, pH 8.0	150 µl

*aqueous solution titrated to pH 7.0 with KOH.

STORAGE

All components should be stored at -20°C.

ADDITIONAL MATERIALS REQUIRED

- DNA template, customer provided (*see* Important Notes)
- Ethanol (70% and 96%)
- Phenol, Tris-saturated, pH 7-8
- Chloroform
- Sterile, disposable plastic ware and RNase-free pipette tips
- Latex gloves, powder free

DESCRIPTION

The TranscriptAid™ T7 High Yield Transcription Kit is designed for high yield *in vitro* transcription from DNA templates containing T7 RNA Polymerase promoter. The kit contains reagents for 50 reactions of 20 µl. Depending on transcript length, each reaction yields approximately 150 µg RNA from 1 µg template in 2 hours (Fig.1), 10 times greater than it is achievable in conventional *in vitro* transcription reactions. The reaction can be scaled-up to produce milligram amounts of full-length RNA.

The kit provides all components for transcription reaction, transcript loading and analysis on a gel. The TranscriptAid™ Enzyme Mix contains T7 RNA polymerase conveniently premixed with recombinant RiboLock™ Ribonuclease Inhibitor to ensure integrity of RNA transcript. DNase I, RNase-free, is provided for efficient removal of template DNA after transcription reaction. The 2X RNA Loading Dye Solution is included for convenience in RNA loading. The RiboRuler™ RNA Ladder, High Range, ready-to-use aids in RNA sizing and quantification on a gel. NTPs are provided in individual tubes for flexibility in synthesis of non-radioactively labeled probes or capped RNA.

The kit can be successfully used to produce both long and short RNA transcripts. The RNA synthesized is suitable in all applications that require large amounts of RNA, such as *in vitro* translation, antisense RNA and RNAi studies, RNase protection assays, studies of RNA splicing, isolation of RNA binding proteins. Non-radioactively labeled RNA can be used as probes in microarrays, blots or *in situ* hybridization.

Due to large quantities of RNA synthesized with TranscriptAid™ T7 High Yield Transcription Kit, generation of high specific activity radiolabeled probes would require prohibitively large amounts of radiolabeled nucleotide. Fermentas T7 Transcription Kit (#K0411) is a recommended alternative for this application.

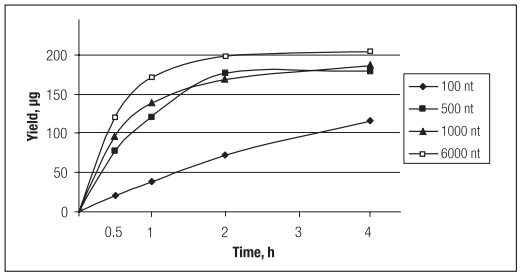


Fig.1. Time course of RNA synthesis

100 nt, 500 nt, 1000 nt and 6000 nt RNA transcripts were generated with TranscriptAid™ T7 High Yield Transcription Kit. Yields of RNA were determined at different transcription reaction time points using Agilent Bioanalyzer 2100.

IMPORTANT NOTES

Avoiding RNase Contamination

All components of the kit have been tested to ensure the lack of contaminating ribonuclease activities. However, an RNase-free working environment and RNase-free solutions are also critical factors for performing successful *in vitro* transcription.

General recommendations to avoid RNase contamination:

- Maintain a separate area, dedicated pipettors and reagents for RNA work.
- Wear gloves when handling RNA and reagents for work with RNA. Change gloves frequently.
- Use sterile RNase-free plastic tubes and pipette tips.
- Treat water and all solutions used for RNA purification and handling with DEPC. Add DEPC to 0.1% (v/v) final concentration, incubate overnight at room temperature and autoclave.
- Keep all kit components sealed when not in use and all tubes tightly closed during transcription reaction.

Template DNA

Double stranded linear DNA with blunt or 5'-protruding ends is a suitable template for *in vitro* transcription reaction. Linearized plasmid DNA, PCR products or cDNA can be used as templates for transcription if they contain a double-stranded T7 promoter region in the correct orientation.

The sequence of T7 promoter is:
5'-taatacgactcactataG*gg-3'.

G* will be the first base of RNA transcript.

The synthesis of the sense or antisense RNA transcript depends on the orientation of the T7 promoter with respect to target sequence. The target sequence must be placed downstream of the T7 promoter for sense RNA and must be inverted for antisense RNA transcription.

Plasmid Templates

Quality

Quality of plasmid DNA affects transcription yield and the integrity of RNA synthesized. The greatest transcription yields are achieved with the highest purity plasmid templates. Plasmids purified by many laboratory methods can be successfully used, if DNA is relatively free of contaminating RNases, proteins and RNA. The GeneJET™ Plasmid Miniprep Kit (#K0502) generates plasmid DNA suitable for transcription.

Linearization

To produce RNA transcripts of a defined length, plasmid DNA is linearized by restriction digestion downstream of the insert to be transcribed (Fig.2). Restriction enzymes which generate blunt ends or 5'-overhangs are preferred. 3'-overhangs have been reported to generate spurious transcripts (1) and generally should be avoided. Otherwise 3'-overhangs can be blunted by Klenow Fragment (#EP0051) or T4 DNA Polymerase (#EP0061) prior to transcription.

Due to high processivity of RNA polymerase, circular plasmid templates generate long heterogeneous RNA transcripts in higher quantities compared to linear templates. Therefore, it is important to achieve complete digestion of circular plasmid to ensure efficient synthesis of defined length transcripts.

After linearization it is recommended to purify template DNA by phenol/chloroform extraction:

1. Extract with an equal volume of 1:1 phenol/chloroform mixture, and then twice with equal volume of chloroform.
2. Precipitate the DNA by adding 1/10th volume of 3 M Sodium Acetate Solution, pH 5.2, and two volumes of ethanol. Incubate at -20°C for at least 30 min and collect the pellet by centrifugation.
3. Remove the supernatant and rinse the pellet with 500 µl of 70% ethanol.
4. Resuspend the pellet in DEPC-treated water (#R0603).

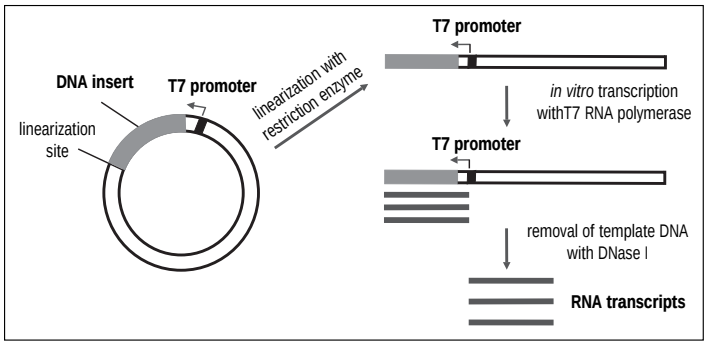


Fig. 2. *In vitro* transcription from linearized plasmid template.

PCR Templates

PCR products can be transcribed directly from the PCR mixture. T7 RNA Polymerase promoter should be located upstream of the sequence to be transcribed. Prior to transcription, an agarose gel electrophoresis of the PCR product is recommended to evaluate the specificity and yield. 2-5 µl of PCR mixture can be directly used in 20 µl of *in vitro* transcription reaction. Visit www.fermentas.com for Fermentas products for PCR.

HIGH YIELD *in vitro* TRANSCRIPTION PROTOCOLS

- Thaw all frozen reaction components, mix and centrifuge briefly to collect all drops.
- Keep TranscriptAid™ Enzyme Mix and nucleotides **on ice**.
- Keep the 5X TranscriptAid™ Reaction Buffer at **room temperature**.
- Combine equal volumes of the four NTP solutions in one tube for convenience and reduction of pipetting steps. If the kit will be used for generation of labeled or capped RNA transcripts, keep nucleotides in separate tubes.

High Yield *in vitro* Transcription

❶ Combine the following reaction components at room temperature in the order given:

Component	Amount
DEPC-treated water	to 20 µl
5X TranscriptAid™ Reaction Buffer	4 µl
ATP/CTP/GTP/UTP mix*	8 µl
Template DNA	1 µg**
TranscriptAid™ Enzyme Mix	2 µl
Total volume	20 µl

* Equal volumes of the four provided NTP solutions combined in one tube.
** For short transcripts (≤100 nt) use 2 µg of template.

❷ Mix thoroughly, spin briefly to collect all drops and incubate at 37°C for 2 h. For short (≤100 nt) transcripts incubate 4-8 h at 37°C.

❸ *Optional:* To remove template DNA add 2 µl of DNase I, RNase free, mix and incubate 15 min at 37°C. Add 2 µl of 0.5 M EDTA, pH 8.0, and incubate at 65°C for 10 min.

Proceed with evaluation of reaction products or purification of RNA transcripts (*see* p.10).

Synthesis of Non-Radioactively Labeled RNA Probes

The recommended molar ratio of modified UTP (Biotin-, Fluorescein-, Digoxigenin- or Aminoallyl-UTP) to standard UTP is 1:3.

❶ Combine the following reaction components at room temperature in the order given:

Component	Amount
DEPC-treated water	to 20 µl
5X TranscriptAid™ Reaction Buffer	4 µl
ATP 100 mM	2 µl (10 mM final concentration)
CTP 100 mM	2 µl (10 mM final concentration)
GTP 100 mM	2 µl (10 mM final concentration)
UTP 100 mM	1.5 µl (7.5 mM final concentration)
modified-UTP*, 50 mM	1 µl (2.5 mM final concentration)
Template DNA	1 µg**
TranscriptAid™ Enzyme Mix	2 µl
Total volume	20 µl

*Biotin-UTP, Fluorescein-UTP, Dioxigenin-UTP or Aminoallyl-UTP (#R1091).
** For short transcripts (≤100 nt) use 2 µg of template.

❷ Mix thoroughly, spin briefly to collect all drops and incubate at 37°C for 2 h. For short (≤100 nt) transcripts incubate 4-8 h at 37°C.

❸ *Optional:* To remove template DNA add 2 µl of DNase I, RNase free, mix and incubate at 37°C for 15 min. Add 2 µl 0.5 M EDTA, pH 8.0, and incubate at 65°C for 10 min.

Note

- Modified ribonucleotides reduce transcription efficiency, therefore lower transcription yields should be expected compared to transcription using unmodified UTP. In addition, the transcripts with incorporated modified ribonucleotides have reduced electrophoretic mobility due to higher molecular weight.
- For subsequent detection of Biotin-labeled probes Biotin Chromogenic Detection Kit (#K0661) and SensiBlot™ Plus Nylon Membrane (#M1002) are recommended. For hybridization protocols, please visit www.fermentas.com.
- Non-radioactively labeled probes are stable for approximately a year when stored at -20°C. Avoid repeated freezing and thawing of the labeled probe.

Synthesis of Capped RNA

The recommended ratio of cap analog to GTP is 4:1.

❶ Combine the following reaction components at room temperature in the order given:

Component	Amount
DEPC-treated water	to 20 µl
5X TranscriptAid™ Reaction Buffer	4 µl
ATP, 100 mM	1.5 µl (7.5 mM final concentration)
CTP, 100 mM	1.5 µl (7.5 mM final concentration)
UTP, 100 mM	1.5 µl (7.5 mM final concentration)
GTP*, **30 mM**	1 µl (1.5 mM final concentration)
Cap analog, 40 mM	3 µl (6 mM final concentration)
Template DNA	1 µg
TranscriptAid™ Enzyme Mix	2 µl
Total volume	20 µl

* For convenience in reaction set-up, prepare 10 µl of 30 mM GTP solution by combining 3 µl of provided 100 mM GTP solution and 7 µl of DEPC-treated water.

❷ Mix thoroughly, spin briefly to collect all drops and incubate at 37°C for 2 h.

❸ *Optional:* To remove template DNA add 2 µl of DNase I, RNase free, mix and incubate at 37°C for 15 min. Add 2 µl 0.5 M EDTA, pH 8.0, and incubate at 65°C for 10 min.

CONTROL REACTION

The Control Template DNA is a 5066 bp linearized plasmid which codes for 2223 nt runoff transcript.

❶ Combine the following reaction components at room temperature in the order given:

Component	Amount
DEPC-treated water	4 µl
5X TranscriptAid™ Reaction Buffer	4 µl
ATP/CTP/GTP/UTP mix*	8 µl
Control DNA (0.5 µg/µl)	2 µl
TranscriptAid™ Enzyme Mix	2 µl
Total volume	20 µl

* Equal volumes of the four provided NTP solutions combined in one tube.

❷ Mix thoroughly, spin briefly to collect all drops and incubate at 37°C for 2 h.

Evaluate the integrity, length and yield of the transcript on agarose gel:

- Dilute 5 µl of control reaction product with 195 µl of DEPC-treated water (40-fold).
- Mix 3 µl of diluted sample with 3 µl of 2X RNA Loading Dye Solution, heat the sample 10 min at 70°C and chill on ice prior to loading.
- Run the sample on a 1% agarose gel with ethidium bromide along with RiboRuler™ RNA Ladder, High Range, ready-to-use.

The control reaction should yield 140-170 µg of a defined 2223 nt RNA transcript in 2 hours.

PURIFICATION OF RNA TRANSCRIPTS

If template DNA will interfere with the downstream application of the RNA transcript, it should be removed by DNase I digestion directly after the transcription reaction.

For removal of all proteins and most of the free nucleotides, phenol:chloroform extraction and ethanol precipitation of RNA transcripts is the preferred method.

1. To 20 µl reaction mixture add 115 µl of DEPC-treated water and 15 µl of 3 M Sodium Acetate Solution, pH 5.2. Mix thoroughly.
2. Extract with an equal volume of 1:1 phenol/chloroform mixture, and then twice with equal volume of chloroform. Collect the aqueous phase and transfer to a new tube.
3. Precipitate the RNA by adding 2 volumes of ethanol. Incubate at –20°C for at least 30 min and collect the pellet by centrifugation.
4. Remove the supernatant and rinse the pellet with 500 µl of cold 70% ethanol.
5. Resuspend the RNA in 20 µl of DEPC-treated water.
6. Store the RNA at -20°C or -70°C.

EVALUATION OF REACTION PRODUCTS

Quantification by UV Light Absorbance

The easiest way to determine RNA concentration is to measure the ultraviolet light absorbance at 260 nm wavelength. Dilute an aliquot of the reaction 1:300 to obtain an absorbance reading in the linear range of a spectrophotometer. For single-stranded RNA, when $A_{260} = 1$, RNA concentration is 40 µg/ml. The RNA yield can be calculated as follows:

$$A_{260} \times 300 \text{ (dilution factor)} \times 40 = \mu g/ml \text{ RNA.}$$

Note. Unincorporated nucleotides and template DNA in the mixture will interfere with the reading. Therefore, for precise quantification it is advisable to remove template and nucleotides from transcription mixture (see “purification of RNA transcript” above).

Sizing and Quantification on Agilent 2100 Bioanalyzer

The Agilent 2100 bioanalyzer can be used for evaluation of the integrity and quantity of an RNA sample. The bioanalyzer separates RNAs according to size by capillary electrophoresis. It requires less RNA for analysis compared to gels. The RNA transcript analyzed should appear as a distinct, sharp peak on the electropherogram.
Follow the manufacturer instructions when using bioanalyzer and RNA LabChip®.

Sizing and Quantification by Agarose Gel Electrophoresis

To evaluate transcript length, integrity and quantity, an aliquot of the transcription reaction should be run on an appropriate native or denaturing (*see* Recipes) agarose gel or polyacrylamide gel.

Transcript length	Recommended gel
>500 nt	1% agarose
100 – 500 nt	2% agarose or 4-5% denaturing polyacrylamide gel
50 – 100 nt	10% denaturing polyacrylamide or 2-3% agarose gel
<50 nt	20% denaturing polyacrylamide or 3-4% agarose gel

- Use only fresh electrophoresis buffers and freshly poured gels.
- Use clean electrophoresis chambers. For RNA gel analysis, avoid electrophoresis tanks, which were used for DNA miniprep analysis since DNA minipreps often contain RNase A.
- 2X RNA Loading Dye Solution contains ethidium bromide therefore for RNA visualization on denaturing formaldehyde gels gel staining is not required.
- For native gels, add 0.5 µg/ml of ethidium bromide to the agarose gel and to the running buffer.
- Use RiboRuler™ RNA Ladder, High range, ready to-use (Fig.3), provided with the kit for the sizing and approximate quantification of the transcript.

Recommendations for RNA Sample Loading

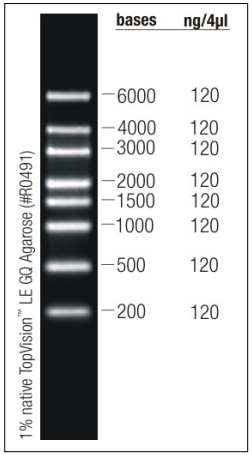
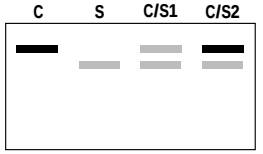


Fig. 3. RiboRuler™ RNA Ladder, High Range, ready-to-use.

1. Dilute RNA transcript 20-40-fold with DEPC-treated water to final concentration of 0.1-0.5 µg/µl.
2. Mix 2-4 µl (0.5-1 µg RNA) of diluted sample with an equal volume of 2X RNA Loading Dye Solution.
3. Heat 10 min at 70°C. Heat an aliquot of the RiboRuler™ RNA Ladder, High Range, ready-to-use in parallel.
4. Chill samples and ladder on ice for 3 min and spin briefly prior to loading onto gel.
5. Use 1 µl of prepared sample loading mixture per 1 mm of gel lane width (4-8 µl depending on well size).
6. Run RiboRuler™ RNA Ladder, High Range in parallel with your samples for sizing and approximate quantification of the transcript. Use 0.5 µl of the ladder per 1 mm of the gel lane width.
7. Run the gel at 5 V/cm, visualize and document.

TROUBLESHOOTING

Problem	Cause and Solution
Low yield of RNA	If the sample template generates RNA transcript of considerably lower yield compared to control template, it is recommended to evaluate experimental template in the mixing experiment. Modify control reaction described in p. 9 by adding equal amount of experimental template to the control template and adjusting the volume of water. Evaluate the transcript on agarose gel as described in p. 10:  **Fig. 4. Evaluation of mixing experiment results** **C** – control template **S** – sample template **C/S1** – mixture of **C** and **S**: control reaction inhibited by sample template solution **C/S2** – mixture of **C** and **S**: control reaction not inhibited by sample template **1. If control reaction is not inhibited by sample template solution (Fig.4. C/S2), it indicates:** **a. Insufficient amount of template.** Low amounts of template produce significantly lower yields of RNA transcript. RNA and chromosomal DNA, present in DNA template preparation may interfere with UV absorbance readings and may lead to misinterpretation of template DNA amount. Therefore check the DNA template both by UV absorbance for amount and by gel electrophoresis for correct size and integrity. **b. Template DNA lost due to precipitation during the reaction assembly**. If the reaction is assembled on ice or in the incorrect order, DNA may precipitate in the presence of spermidine in the reaction buffer. Water should be added first to the transcription reaction. **2. If control reaction was inhibited by sample template (Fig.4. C/S1), it indicates reaction inhibitors in template DNA solution.** Template DNA may contain residual SDS, EDTA, salts and RNases. Repurify template by phenol/chloroform extraction and ethanol precipitation, expect A_{260}/A_{280} ratio of 1.8-2.0. To remove EDTA and salts, wash the pellet with 70% cold ethanol (*see* "Plasmid templates"p. 4).

Problem	Cause and Solution
Lower yields of short transcript	High yields of short transcripts (≤100 nt) are achieved by increasing the amount of template and extending incubation time. Use 2 µg of template and prolong reaction time to 4-8 hours. Do not incubate for more than 8 hours.
RNA transcript of incomplete length	**RNA polymerase may recognize some sequences as terminators.** Perform transcription reaction at lower temperatures, for example at 30°C. Sometimes this can increase the length of transcript, whereas at lower temperatures the yield can be decreased. **GC rich template.** Incubation at 42°C or use of single-stranded binding (SSB) protein has been reported to improve yield and length of transcript reaction from templates with secondary structures (2).
RNA transcript of larger size	**Incomplete denaturation of RNA sample in the gel.** Due to secondary structures RNA may run aberrantly on a native gel. On a denaturing gel such transcripts usually migrate as single bands of the correct size. **Incomplete cleavage of template plasmid DNA.** Even small amounts of undigested circular DNA can produce large amounts of long transcripts. Check template for complete digestion and, if required, additionally digest with restriction enzyme. **3'-overhangs at DNA template ends.** Avoid restriction enzymes generating ends of this type for plasmid linearization, or blunt 3'-overhangs with Klenow Fragment (#EP0051) or T4 DNA Polymerase (#EP0061) before use in transcription.
RNA transcript smearing on denaturing agarose gel	**DNA template is contaminated with RNase.** During preparation plasmid DNA templates often get contaminated with RNases that can affect the length and yield of RNA synthesized (a smear below the expected transcript length). If using commercial kits, such as GeneJET™ Plasmid Miniprep Kit, omit RNase A from plasmid preparation solutions and use DEPC-treated water (#R0603) for plasmid elution. If RNase A is pre-included into purification buffers, perform phenol/chloroform extraction after plasmid DNA linearization, then ethanol precipitate DNA and dissolve in DEPC-treated water (*see* "Plasmid templates" p. 4)

RECIPES

10X MOPS buffer

0.4 M MOPS (pH 7.0)
0.1 M Sodium Acetate
0.01 M EDTA (pH 8.0)

Denaturing formaldehyde gel (1%)

1. Add 1 g of agarose powder (TopVision™ LE GQ agarose, #R0491) to 72 ml of deionized water and melt.
2. Add 10 ml of freshly prepared 10X MOPS buffer (*see* above) and mix.
3. Let cool down to 60°C and add 18 ml of fresh formaldehyde 37% (in a fume hood), and mix.
4. Pour the gel.
5. Place the gel into an electrophoresis apparatus containing fresh 1X MOPS buffer.

QUALITY CONTROL

All components of the kit are tested in a functional control assay as described in the manual. A control 20 µl reaction, which contains 1 µg of control template coding for 2.2 kb RNA transcript, yields 140-170 µg of intact RNA after a 2 h incubation.

RELATED PRODUCTS

Product	Amount	Catalog #
RiboRuler™ RNA Ladder, Low Range	50-100 applications	SM1831
RiboRuler™ RNA Ladder, Low Range, ready-to-use	50-100 applications	SM1833
RiboRuler™ RNA Ladder, High Range	50-100 applications	SM1821
RiboRuler™ RNA Ladder, High Range, ready-to-use	50-100 applications	SM1823
2X RNA Loading Dye Solution	1 ml	R0641
DEPC-treated Water	5 x 1 ml	R0603
	30 ml	R0601
TopVision™ LE GQ Agarose	100 g	R0491
TopVision™ LM GQ Agarose	25 g	R0801
50X TAE Buffer	1 liter	B49
10X TBE buffer	1 liter	B52
0.5 M EDTA, pH 8.0	5 x 1 ml	R1021
Aminoallyl-UTP, 50 mM	2.5 µmol	R1091
RiboLock™ Ribonuclease Inhibitor	2500 u	EO0381
	4 x 2500 u	EO0382
DNase I, RNase free (1 u/µl)	1000 u	EN0521
DNase I, RNase free (50 u/µl)	1000 u	EN0523
Klenow Fragment	300 u	EP0051
	1500 u	EP0052
T4 DNA Polymerase	100 u	EP0061
	500 u	EP0062
GeneJET™ Plasmid Miniprep Kit	50 preps	K0502
	250 preps	K0503
T7 Transcription Kit	10 reactions	K0411
	30 reactions	K0412
Biotin Chromogenic Detection Kit	10 reactions	K0661
	30 reactions	K0662
SensiBlot™ Plus Nylon Membrane	30 cm x 3 m	M1002
pTZ19R DNA	50 µg	SD0141

SAFETY INFORMATION

RiboRuler™ RNA Ladder, High Range, ready-to-use;
2X RNA Loading Dye Solution

T Toxic

Hazard-determining component of labeling: **formamide**

Risk phrases

R61 May cause harm to the unborn child.

Safety phrases

S53 Avoid exposure - obtain special instructions before use.
S20 When using do not eat or drink.
S23 Do not breathe gas/fumes/vapour/spray.
S36/39 Wear suitable protective clothing and eye/face protection.
S45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
S60 This material and its container must be disposed of as hazardous waste.

References

1. Schenborn, E.T. and Mierendorf, R.C., Nucl. Acids Res.,13, 6223-6236, 1985.
2. Aziz, R.B. and Soreq, H., Nucl. Acids Res., 18, 3418, 1990.

Trademarks

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively for research purposes and *in vitro* use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.fermentas.com for Material Safety Data Sheet of the product.

(1) Revised 27.03.2007