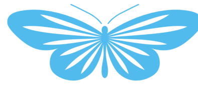

Manual

TranScriba™ noGenome™ Kit

Kit for first-strand cDNA synthesis with efficient genomic DNA removal. Contains RNAse inhibitor and standard primers.

catalog #	size
4000NG-20	20 reactions in 20 µl
4000NG-100	100 reactions in 20 µl

For research use only.

Guarantee
A&A Biotechnology provides a guarantee on this product.
The company does not guarantee the correct performance of this kit in the event of:

- not adhering to the supplied protocol
- use of not recommended equipment or materials
- use of other reagents than recommended or which are not a component of the product
- use of expired or improperly stored product or its components

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Description

The TranScriba^{TM} noGenome™ Kit utilizes minimum hands-on time and effort compared to already existing DNA removal protocols combined with reverse transcription. It consists of two consecutive steps: efficiently getting rid of genomic DNA and first-strand cDNA, high yield synthesis.

The RNA sample is briefly incubated in the noGenome™ buffer to effectively remove genomic DNA. After genomic DNA elimination, the pure RNA sample is ready for reverse transcription. The reverse transcription may take from 15 to 60 min depending on target RNA features and template quality.

TranScriba™ reverse transcriptase has a high affinity for RNA and is optimized for efficient and sensitive cDNA synthesis from 10 pg to 1 µg of RNA. This high affinity, in combination with thermostability and reaction buffer, enables high cDNA yields, even from templates with high GC-content or complex secondary structures.

Contents

component	20 reactions	100 reactions	storage
TranScriba™ reverse transcriptase	100 µl	500 µl	-20 °C
5x reaction buffer	100 µl	500 µl	-20 °C
noGenome™ buffer	100 µl	300 µl	-20 °C
RNAse inhibitor	20 µl	60 µl	-20 °C
dNTP's mix	50 µl	250 µl	-20 °C
oligo $(dT)_{18}$ primer	30 µl	125 µl	-20 °C
dN-hexamer primer	30 µl	125 µl	-20 °C
sterile water	2 x 1.5 ml	4 x 1.5 ml	-20 °C

Additional equipment and reagents

- PCR tubes
- Ice
- Heat block or water bath
- Vortex
- Microcentrifuge
- Thermocycler

Notes

- Wear a lab coat and gloves and set up all reactions on ice to minimize the risk of RNA degradation.
- If there is any precipitate visible in the 5x reaction buffer or noGenome™ buffer after thawing, dissolve them by vortexing. If necessary, briefly incubate at 37 °C until the precipitates disappear.
- Repeated, 7x thawing and freezing cycles do not influence the quality of the product.

Before you begin

Thaw at room temperature:

- template RNA
- suitable primer
- noGenome™ buffer
- TranScriba™ reverse transcriptase
- 5x reaction buffer
- dNT';s mix
- RNAse inhibitor
- sterile water

Mix each solution by inverting the tube a few times. Centrifuge briefly and keep on ice.

Master mixes preparation

- Prepare a volume of master mixes 10% greater than required for the total number of reactions to be performed.
- Always prepare fresh master mixes. Do not store any leftovers.

noGenome™ master mix

(gDNA removal reaction mix)

1. Prepare **noGenome™ master mix** according to the table below:

component	amount per reaction
noGenome™ buffer	2 µl
sterile water	8 µl

2. Mix and dispense **10 µl** to PCR tubes for single reactions. Keep on ice.

TranScriba™ master mix

(cDNA synthesis reaction mix)

1. Prepare the **TranScriba™ master mix** according to the table below:

component		amount per reaction
5x reaction buffer		4 µl
RNAse inhibitor		0.5 µl
dNTP's mix		2 µl
Primer	**oligo $(dT)_{18}$**	1 µl
	or	
	dN-hexamer	1 µl
	or	
	gene-specific	15-25 pmol
TranScriba™ reverse transcriptase		4 µl
sterile water		up to 13 µl

2. Mix and dispense **13 µl** to PCR tubes for single reactions. Keep on ice.

Protocol

1. Add **4 µl** of **template RNA** to the **noGenome™ master mix** on ice.

   Note. The protocol is for use with 10 pg to 1 µg of RNA. This amount corresponds to the total amount of RNA present, including any rRNA, mRNA, viral RNA, and carrier RNA present. Regardless of the primers used or cDNA analyzed. If using >1 µg of RNA, scale up the reaction linearly.

2. Mix and incubate for **2 min** at **42 °C.** Keep on ice.

   Attention! Do not incubate at 42 °C longer than 10 min.

3. Transfer **7 µl** of the **noGenome™ master mix** with **template RNA** to a PCR tube with **TranScriba™ master mix** on ice. Mix gently by pipetting. Close the tubes and centrifuge briefly.

   Note. We suggest the remaining 7 µl of the noGenome™ master mix with template RNA be added to negative reaction control without TranSrciba™ reverse transcriptase. This will serve as a control for gDNA removal reaction efficiency in the downstream PCR.

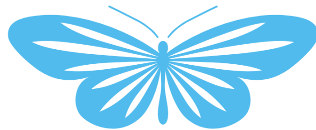
4. Transfer the tubes to the preheated cover PCR machine and run the suitable program according to the guidelines given below and in point 5. of the protocol.

primer type	temperature	time
oligo $(dT)_{18}$	42 °C	15-60 min
gene-specific	42 °C	15-60 min
dN-hexamer	25 °C 42 °C	5 min followed by 15-60 min

   Note: Optimal reverse transcription time depends on a specific cDNA product to be elongated.

5. Terminate the reaction by incubating for **5 min** at **70 °C**.

   The cDNA first strand can be used directly in any PCR application or stored at -20 °C.



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