A&A BIOTECHNOLOGY
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Manual

TranScriba™ Kit

Kit for first strand cDNA synthesis. Contains RNAse inhibitor and standard primers.

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

For research use only.

Guarantee

A&A Biotechnology provides guarantee on this product.
The company does not guarantee 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

Description

TranScriba™ Kit is a complete set of reagents for first strand cDNA synthesis from mRNA or RNA templates.
The kit utilizes a recombined high processivity mutant MMLV reverse transcriptase with its intrinsic low RNAse H activity and optimal DNA polymerase activity within 37-42 °C temp. The RNA template is protected by a recombinant RNAz inhibitor.
Kit also contains both oligo(dT)$_{18}$ and random hexamer primers. Gene specific primers may also be used with the kit to prime cDNA synthesis from specific RNA sequences.

Contents

	4000-20	4000-100	storage
TranScriba™ reverse transcriptase	100 µl	500 µl	-20 °C
RNAse inhibitor	20 µl	60 µl	-20 °C
5x reaction buffer	100 µl	500 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

Notes

- Before use all solutions should be thoroughly thawed and mixed by inverting the tube.
- Use certified nuclease-free labware.
- Work sterile and use all RNA lab work precautions, wear gloves and change them whenever appropriate.
- Up to 7x repeated freeze-thaw cycles do not influence the activity of this product.

RNA template

RNA purity and integrity are essential for successful synthesis of full-length cDNA. The total RNA isolated by standard methods is compatible with TranScriba™ Kit.
For the best results we recommend using the kit for RNA isolations series Total RNA Mini (cat. # 031-25, 031-100).
For all PCR applications RNA must be free of DNA. To remove DNA contamination treat RNA template prior to the reverse transcription with RNA Clean-Up Kit (cat # 039-25C, 039-100C) or Total RNA Zol-Out™ D (cat # 043-25, 043-100).

Ribonuclease contamination

All components are rigorously tested to ensure RNAse free standard. RNAse inhibitor protects against RNAses that could be introduced when using the kit. It completely prevents the degradation of RNA template up to a concentration of 0.1 ng of RNAse per 1 µg of reverse transcription reaction mixture.

Typical protocol for first strand cDNA synthesis

1. Thoroughly thaw all the components, gently mix by inverting the tube, briefly centrifuge. Place the tube on ice.

2. Place a sterile PCR tube on ice and add:

component	amount
RNA template total RNA / mRNA	 0.1-5 µg / 10 ng-0.5 µg
primer oligo(dT)$_{18}$ or dN-hexamer or gene-specific primer	 1 µl 15-25 pmol
sterile water	up to 9.5 µl

3. Close the tube. Gently mix the sample and briefly centrifuge. Incubate for 5 min at 65 °C to denature the RNA template. Cool on ice immediately, centrifuge and place on ice again.

4. Open the tube and add remaining components in the following order:

component	amount
5x reaction buffer	4 µl
RNAse inhibitor	0.5 µl
dNTP's mix	2 µl
TranScriba™ reverse transcriptase	4 µl

5. Close the tube. Gently mix the sample and briefly centrifuge.

6. Incubate the mixture depending on the primer used:

   - **oligo(dT)$_{18}$** or **gene-specific** primer: 30-60 min at 42 °C
   - **dN-hexamer:** 5 min at 25 °C, next 60 min at 42 °C

7. Terminate the reaction by heating for 5 min at 70 °C.
   Obtained the cDNA first strand can be directly used in any PCR application or stored at -20 up to -70 °C.

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