AnyGenes®

Efficient technologies for signaling pathways

StaRT Reverse Transcription Kits
User Guide

- ✓ Cat # StaRT-10
- ✓ Cat # StaRT-25
- ✓ Cat # StaRT-50

Compatible with all AnyGenes® SignArrays products

For research use only

Store at -20°C

2018

Summary

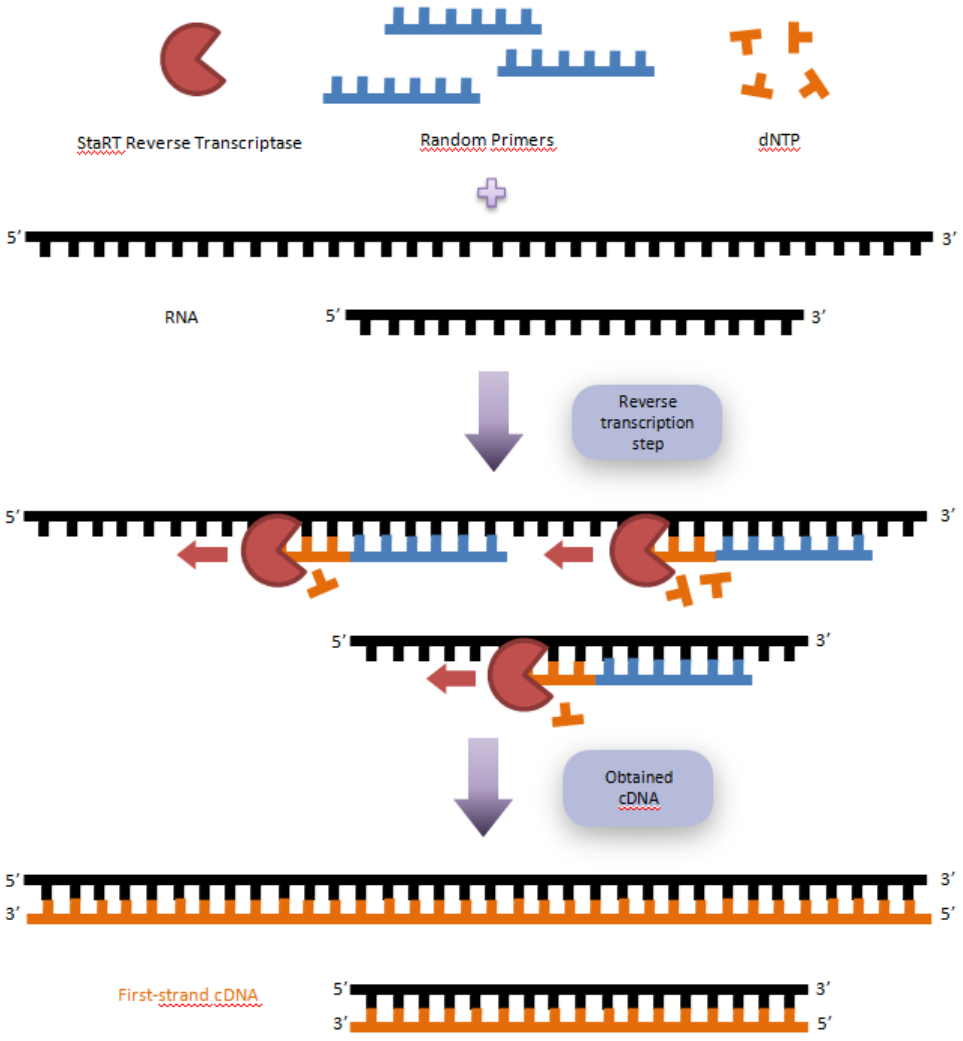
I. Product information

1) Introduction

The **StaRT Reverse Transcription kit** is an optimised and complete solution to synthetize first-strand cDNA from total RNA, ideal for quantitative real-time PCR (qPCR) applications and fully compatible with the AnyGenes® SignArrays® system and SpeAmp^{n} cDNA pre-amplification kits.

You can perform high-throughput transcriptomic analysis with only small amounts of biological material (5-50ng RNA), used in combination with the AnyGenes SpeAmp^{n} system.

Principle :



The StaRT Reverse Transcription kit has been **optimised and experimentally validated** thanks to our strict quality control policy in order to give **highly reliable and reproducible results in qPCR applications**.

It contains :

- **StaRT Reverse Transcriptase** (50U/µl), a highly efficient *Moloney Murine Leukemia Virus* Reverse Transcriptase (M-MLV)

- **StaRT Buffer (10X)**, which is an optimized buffer allowing to ensure the best Reverse Transcription (RT) performance

- **Random Primers (10X),** allowing Reverse Transcription of total RNA, even long non-coding RNA (LncRNA)

- and **dNTP mix (10X)**.

The StaRT Reverse Transcription step requires only the preparation of the StaRT mix, then the addition of your RNA sample and incubation. The performance of AnyGenes® StaRT Reverse Transcription kit has been carefully designed to provide you the best results in downstream transcriptomic applications. For any details, see www.anygenes.com.

✓ **Quality Control**

As part of our routine quality assurance program, all AnyGenes® products are monitored to ensure the highest levels of performance and reliability.

2) Intended use & licencing

For molecular biology research use only. This kit is not intended for diagnosis, prevention or therapeutic applications. AnyGenes® will be not responsible of the misuse of their products.

Purchase of AnyGenes® kits does not include or provide licence with respect to any patents owned by other companies.

3) Kit contents

The StaRT Reverse Transcription kit is comprised of :

- StaRT Reverse Transcriptase (50U/µl)
- StaRT Buffer (10X)
- Random Primers (10X)
- and dNTP mix (10X).

These products are available in several formats compatible with all of our SignArrays® systems :

Catalog Ref :	Contents
StaRT-10	**StarRT Reverse Transcriptase 50U/µl** (1 x 10 µl) + **StaRT Buffer (10X)** (1 x 20 µl) + **Random Primers (10X)** (1 x 20 µl) + **dNTP mix (10X)** (1 x 20 µl) + **PCR grade H_2O** (1 x 1.5 mL)
StaRT-25	**StarRT Reverse Transcriptase 50U/µl** (1 x 25 µl) + **StaRT Buffer (10X)** (1 x 50 µl) + **Random Primers (10X)** (1 x 50 µl) + **dNTP mix (10X)** (1 x 50 µl) + **PCR grade H_2O** (1 x 1.5 mL)
StaRT-50	**StarRT Reverse Transcriptase 50U/µl** (1 x 50 µl) + **StaRT Buffer (10X)** (1 x 100 µl) + **Random Primers (10X)** (1 x 100 µl) + **dNTP mix (10X)** (1 x 100 µl) + **PCR grade H_2O** (1 x 1.5 mL)

For more product information, please visit **www.anygenes.com** or contact us at **technical@anygenes.com**

4) Storage & stability

Upon receipt, store StaRT kits at -20°C until their use. These storage conditions guarantee a long-term storage of AnyGenes® products for a minimum period of 12 months after their receipt. Moreover, in order to guarantee the stability of these products, avoid repeated freezing and thawing cycles. If small volumes of reagents are frequently required, we recommend to stock alicots at -20°C.

5) Additional reagents and equipment required

A) Reagents :

- total RNA
- Ultra-pure & sterile « nuclease, *RNAse, DNAse free »* H_2O (*supplied with StaRT kits*)

Caution : Do not use DEPC H_2O !!!

B) Material :

- Thermal Cycler Instrument (with sufficient ramp rate (> 1°C/s))
- PCR plates centrifuge, vortex mixer and mini-centrifuge
- “nuclease, RNase, DNase free” tips PCR plates, sealing foils and tubes
- Pipettes for reaction mix preparation and dispensing

For more product information, please visit **www.anygenes.com** or contact us at **technical@anygenes.com**

II. Protocol

1) Before you start...

To obtain reliable and reproducible results and avoid contamination and false-positive signals, it is important and necessary to follow Good Laboratory Practices.

Caution: Ensure of the RNA purity and integrity before starting all your experiments. AnyGenes cannot guarantee optimal results with poor quality RNA (poor integrity (RIN <7) or contaminated with solvants or proteins (A260/A280 ratio non included between 1.8 and 2)).

2) Procedure

1) Prepare the work area (highly recommended under workstation) by carefully cleaning all material and areas with a suitable detergent and then decontaminating the workstation through exposure to UV.

2) Thaw AnyGenes® **Perfect Master Mix**, **Prim^{nx} reagents** and your **RNA samples** 20 minutes before use, in order that slowly reaches room temperature. You can also work with your samples on ice.

3) Prepare your RNA **in a 10µl final volume** by diluting **1 or 2 µg of RNA** (according to your downstream experiments) with Ultra-pure & sterile « nuclease, *RNAse, DNAse free »* H_2O in new 0.5 mL tubes.

   NB : Reverse Transcription of **1 µg RNA** is necessary to perform qPCR in **1 entire SignArray® 96**
   Reverse Transcription of **2 µg RNA** is necessary to perform qPCR in **1 entire SignArray® 384**

4) Mix your RNA samples by pipetting, and incubate them 5 minutes at 65°C to denature RNA.

5) Meanwhile, briefly centrifuge tubes and reagents and prepare the Reverse Transcription reaction mix in a 1.5 ml tube, according to the following table :

Reagents	For 20 µl final reaction volume	
	Volumes / reaction	For n reactions
StaRT Buffer 10X	2 µl	n x 2 µl
Random Primers 10X	2 µl	n x 2 µl
dNTP mix 10X (40 mM)	2 µl	n x 2 µl
Ultra-pure H_20	3 µl	n x 3 µl
StaRT Reverse Transcriptase 50U/µl	1 µl	n x 1 µl
Total StaRT Reaction Mix Volume	10 µl per reaction	n x 10 µl per reaction to dispense

NB : Prepare your mix with 5-10% of excess volume to prevent loss of reagents during pipetting.

6) Briefly mix the **StaRT Reaction Mix** by pipetting.

7) Recover your RNA tubes.

8) Dispense 10µl of **StaRT Reaction Mix** per RNA sample.

	Volumes / reaction
Diluted RNA template (until 2 µg)	10 µl
StaRT Reaction Mix	10 µl
Total Reaction Volume	**20 µl per well or tube**

9) Mix thoroughly with a pipette or briefly centrifuge each tube.

10) Program your Thermal Cycler to perform your Reverse Transcription as below :

StaRT steps	**Time**	**Temperature**
1st step	10 minutes	25°C
2nd step	120 minutes	37°C
3rd step	5 minutes	85°C
4th step *(optional)*	∞	4°C

11) Start the Reverse Transcription run.

12) Once the run is complete, release the plate or tubes of your Thermal Cycler.

13) Store your cDNA at 4°C.

To continue with the SignArrays system :

- Dilute your cDNA just before performing your qPCR SignArrays, according to the instructions of the SignArrays 96 or 384 Handbooks.

III. Additional Informations

For further information, please contact AnyGenes® technical support via the following email address : technical@anygenes.com

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