

## TECHNICAL DATA SHEET

# FastSCRIPT™ cDNA Synthesis Kit

Catalog Numbers: 31-5300-0025R  
31-5300-0100R

## PRODUCT INFORMATION

**Contents:** 31-5300-0025R (25 rxns)  
FastSCRIPT™ cDNA Synthesis Mix (5X): 1 x 100 µL  
M-MLV RTase / RNase Inhibitor Mix (20X): 1 x 25 µL

31-5300-0100R (100 rxns)  
FastSCRIPT™ cDNA Synthesis Mix (5X): 4 x 100 µL  
M-MLV RTase / RNase Inhibitor Mix (20X): 4 x 25 µL

**Use By:** 6 months from date of receipt

## DESCRIPTION

Tonbo's FastSCRIPT™ cDNA Synthesis Kit contains a proprietary M-MLV Reverse Transcriptase (RT) molecule blended with an optimized RNase inhibitor, along with a synthesis mix including random hexamers and anchored oligo(dT) primers that have been preoptimized for the generation of cDNA for use in real-time PCR experiments. The kit can be used with 4.0 pg - 4.0 µg total RNA template.

Tonbo's M-MLV RT enzyme can synthesize cDNA at a temperature range of 45–55°C, providing increased specificity, higher yields of cDNA, and more full-length product than other reverse transcriptases. Notably, it is not significantly inhibited by ribosomal and transfer RNA, making it ideal to synthesize cDNA from total RNA. The modified MMLV reverse transcriptase is both thermostable and extremely active.

The reverse transcriptase enzyme, buffer system and combination of random hexamers with anchored oligo(dT) primers allow for unbiased, efficient, sensitive cDNA synthesis. The FastSCRIPT cDNA Synthesis Kit can be used with total or poly(A)+ RNA, miRNA and viral RNA targets.

## STORAGE

Store kit at -20°C upon arrival and limit exposure to light. The kit may undergo up to 30 freeze/thaw cycles without loss of activity. When stored correctly this kit will retain activity for up to 6 months. This product can be stored at 4°C for up to 1 month.

## BIOLOGICAL SOURCE

Tonbo's Reverse Transcriptase is a proprietary version of M-MLV RT that has been genetically engineered to reduce RNase H activity and provide increased thermal stability.

## APPLICATION NOTES

**Master Mix:** FastSCRIPT cDNA Synthesis Mix (5X) contains 15 mM MgCl<sub>2</sub>, 5 mM dNTPs, anchored oligo(dT) primers and random hexamers in a buffer containing a proprietary mix of stabilizers and enhancers. Tonbo's cDNA Synthesis Mix has been rigorously developed for optimal success rate, yield and efficiency. We do not recommend introducing additional MgCl<sub>2</sub> or enhancers to the reaction mix.

**Template:** We recommend using between 4.0 pg – 4.0 µg of total RNA per reaction.

**Incubation Temperature:** Incubation for 30 minutes at 42°C should work well for most applications where GC content is below 65%. Temperature can be increased up to 55°C in cases where regions of interest have a high degree of secondary structure (>65% GC).

**Downstream qPCR Reactions:** cDNA generated from this kit can be used at 4.0 µl per 20 µl real-time PCR reaction.

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**REACTION SETUP / QUICK PROTOCOL**

1. Ensure all components are thawed and mixed well. Set up reactions on ice. Refer to Table 1 for reaction preparation - we recommend adding reagents in the order indicated. If preparing multiple reactions, assemble all common components into a master reaction mix. If working with final reaction volumes greater than 20 µl, scale component volumes accordingly.
2. Optional: We recommend including a no-RTase control reaction (do not add M-MLV RTase / RNase Inhibitor Mix) which will act as a control for the presence of contaminating genomic DNA in the reaction. A no-template control (do not add template RNA) can also be set up as a control for the presence of contaminating genomic DNA in the enzyme/primer mixes.
3. As applicable, transfer the recommended volume of master reaction mix, primers and sample template RNA to individual PCR tubes or plates, seal and spin briefly to mix.
4. For the cDNA reaction:
  - a. Incubate at 42°C for 30 minutes.
  - b. Incubate at 85°C for 10 minutes to denature the RTase.

**Table 1. Reaction Preparation**

Reagent	20 µL reaction	Final Concentration	Notes
FastSCRIPT™ cDNA Synthesis Mix (5x)	4.0 µL	1x	
20x RTase Mix	1.0 µL		Add prior to total RNA as RTase Mix contains RNase inhibitor
Total RNA (between 4 pg and 4.0 µg)	X µL		variable
Nuclease free dH <sub>2</sub> O	Up to 20 µL final volume		

**TECHNICAL SUPPORT**

Please provide the following information to [support@tonbobio.com](mailto:support@tonbobio.com) for troubleshooting and technical support:

- Catalog and batch numbers
- Reaction set-up (master mix)
- Cycling conditions
- Amplicon size
- Screen shots of gel images and/or amplification traces
- Detailed description of the issue.

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