

Check the product label for actual catalog number, lot and expiry date.

## HighScriber™ Reverse Transcriptase Mix, 20X

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
RTM0301	10000 u/ 50 r	2 x 25 µl - HighScriber™ Reverse Transcriptase Mix, 20X 2 x 0.25 ml - 5X ALLin™ HighScriber Buffer	Enzyme Mix contains HighScriber™ Reverse Transcriptase at 200 u/µl concentration, Ribonuclease Inhibitor and glycerol. 5X ALLin™ HighScriber Buffer contains MgCl <sub>2</sub> , dNTPs, enhancers, stabilizers.
RTM0305	50000 u/ 250 r	10 x 25 µl - HighScriber™ Reverse Transcriptase Mix, 20X 10 x 0.25 ml - 5X ALLin™ HighScriber Buffer	Enzyme Mix contains HighScriber™ Reverse Transcriptase at 200 u/µl concentration, Ribonuclease Inhibitor and glycerol. 5X ALLin™ HighScriber Buffer contains MgCl <sub>2</sub> , dNTPs, enhancers, stabilizers.

**Storage** In the dark at -20°C.

### APPLICATIONS

- cDNA synthesis of up to 15 kb long transcripts
- Template generation for RT-PCR & RT-qPCR
- cDNA synthesis from complex templates

### PRODUCT DETAILS

The HighScriber™ Reverse Transcriptase Mix is a premium tool for the high efficiency reverse transcription of up to 12-15 kb long cDNA. Mix includes HighScriber™ Reverse Transcriptase and Ribonuclease Inhibitor for save, robust cDNA synthesis and ease of use. HighScriber™ Reverse Transcriptase allows for high detection sensitivity from 1 pg of total RNA. The wide reaction temperature range (38°C - 55°C) ensures efficient cDNA synthesis from complex or GC rich templates. The enzyme uses ssRNA or ssDNA as a template, possesses no detectable Ribonuclease H activity specific to RNA in RNA-DNA hybrids. A highly reduced Ribonuclease H activity allows for transcription of full lengths long transcripts. HighScriber™ Reverse Transcriptase can be used for RACE as it has terminal transferase activity - adds cytosines to 3' ends of cDNA.

### BENEFITS

- Thermostable Reverse Transcriptase blended with Ribonuclease Inhibitor for efficient cDNA synthesis
- High yields of full lengths transcripts up to 12-15 kb
- cDNA synthesis from complex templates at up to 55°C
- High sensitivity detection from 1 pg of total RNA template

### PRODUCT SPECIFICATIONS

The Ribonuclease inhibitor premixed with the RT ensures RNA protection from ribonuclease degradation. Supplied 5X ALLin™ HighScriber Buffer includes everything you need for the cDNA synthesis reaction. To minimize pipetting steps it contains MgCl<sub>2</sub>, dNTPs, enhancers, stabilizers. The only things to add is the template RNA and primer.

- Optimal RT activity is observed at 45-50°C
- RT temperature range 38-55°C
- RT Inactivation at 85°C for 10 min

### Unit Definition

One unit is defined as the amount of enzyme that will incorporate 1 nmol of dTTP into acid-insoluble material in a total reaction volume of 50 µl in 10 minutes at 37°C using poly (rA) oligo (dT)<sub>18</sub> as template.

### PROTOCOL

- RNA is extremely sensitive to degradation by RNases present everywhere. Take care to protect RNA from degradation keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Check the integrity of RNA prior to cDNA synthesis in denaturing agarose gel.
- Include positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- For best results, optimize the template and primer amount.
- Do not exceed the recommended amount of the enzyme mix.
- Perform reaction for 30-50 min, for short transcripts 15-30 min are sufficient.
- Choose optimal reaction temperature in a range of 42-55°C.
- Do not add Ribonuclease Inhibitors and dNTPs, as they are already included in supplied Mix and buffer

#### ✓ Prepare a 20 µl reaction:

5X ALLin™ HighScriber Buffer	4 µl (includes dNTPs)
Oligo dT primer <i>or</i>	0.5 µg <i>or</i>
Random primer <i>or</i>	0.2 µg <i>or</i>
Specific primer	15-20 pmol
Total RNA <i>or</i>	1 pg to 5 µg <i>or</i>
Poly-A mRNA	1 pg to 0.5 µg
Water (PCR Water, WAT0110)	to 19 µl
✓ Mix gently.	
✓ Heat 5 min at 65°C, spin, place on ice for 1 min.	
✓ Incubate 2 min at 42°C for Oligo dT and for Specific primer <i>or for</i>	10 min at 25°C for Random primer to anneal.
✓ Add 1 µl HighScriber™ Reverse Transcriptase Mix, 20X and mix well.	
✓ Incubate 30-50 min at 50°C to synthesize cDNA.	
✓ Inactivate at 85°C for 10 min.	
✓ Store reactions at -20°C or on ice for an immediate use.	
✓ Use 2-5 µl of this reaction mix per 50 µl PCR reaction.	
✓ Use 2 µl of this reaction mix per 20 µl qPCR reaction.	

IN VITRO RESEARCH USE ONLY

#### ORDERING

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