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 0.2 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 $MgCl_2$, dNTPs, enhancers, stabilizers.
RTM0305	50000 u/ 250 r	10 x 25 µl - HighScriber™ Reverse Transcriptase Mix, 20X 10 x 0.2 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 $MgCl_2$, 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

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 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.

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 $MgCl_2$, 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 $(dT)_{18}$ 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 *or* Random primer *or* Specific primer	0.5 µg *or* 0.2 µg *or* 15-20 pmol
Total RNA *or* Poly-A mRNA	1 pg to 5 µg *or* 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 *or for* 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
T: +49 7250 33 13 401
F: +49 7250 33 11 413
order@highQu.com
www.highQu.com

SALES
T: +49 7250 33 13 401
F: +49 7250 33 11 413
sales@highQu.com

TECHNICAL SUPPORT
T: +49 7250 33 13 401
F: +49 7250 33 11 413
tech-support@highQu.com