Accuris qMax™ First Strand cDNA Synthesis Flex Kit

Description

High quality, PCR ready cDNA is quickly generated with the Accuris qMax First Strand cDNA Synthesis Flex Kit. The exceptionally thermostable MMLV- derived qMax High Capacity Reverse Transcriptase combined with a potent RNase inhibitor allows for transcription to take place at higher temperatures (alleviating secondary structure problems) and maintains the integrity of the total RNA. The 5X buffer provides the perfect environment for highly efficient cDNA synthesis and greater yields. The buffer is optimized for superior data accuracy and reproducibility with a wide range of RNA sources.

- -Separate solutions of oligo (dT) primers and random hexamer primers for greater flexibility in assay design.
- -Input range of 10pg to 2µg of total RNA
- -Thermostable, high capacity reverse transcriptase and optimized next generation buffer system provide consistent, high yields of fragments up to 9Kb in length

Upon receipt, immediately store at -20°C. Avoid excessive freeze/thaw cycles. When stored as directed, this product will retain its activity for 12 months from date of receipt.

Limitations of Use

For research purposes only. Not intended for therapeutic or diagnostic use.

Quality Control

Accuris enzymes and reagents are tested under general assay conditions for activity, reproducibility, efficiency, heat activation, sensitivity, and absence of nuclease contamination and nuclease activity. This product is manufactured under a comprehensive quality management system, following ISO 9001:2008 standards.

Accuris is not responsible for consequential or incidental damages, direct or indirect, resulting from use of this product. Accuris guarantees the performance of this product as described when used in accordance with these instructions.

General Guidelines

Buffer

The cDNA Synthesis Kit is supplied with a specially formulated 5X buffer. This buffer has been optimized to work with the RT provided in the kit and contains optimal levels of 5mM dNTPs, 15mM $MgCl_2$, stabilizers and enhancers. High quality cDNA can be produced with maximum efficiency using this buffer and RT. The use of other additives is not required or recommended.

Template

The First Strand cDNA Synthesis Flex Kit works optimally with 10pg to 2.0µg of total RNA or 5pg to 0.5µg of oligo (dT) purified mRNA. If input RNA is in excess of 2.0µg in a 20µl reaction volume, best results will be obtained by increasing the suggested amount of high capacity reverse transcriptase by 1.5 to 2 times.

Reaction conditions

Most reactions can be carried out at a temperature of 42°C for 60 minutes. When working with templates that have a high GC content (above 65%), the temperature should be increased to 45°C to alleviate any problems associated with secondary structure. For random hexaprimers, incubate for 10 minutes at 25°C and then increase the temperature to 42°C for 60 minutes.

Analysis by qPCR

A cDNA synthesis reaction produces enough cDNA for analysis by real time PCR as well as other down stream processing reactions. For a 20µl qPCR reaction, using 2.0 to 4.0µl of the cDNA synthesis reaction product is recommended. cDNA may be stored at 4°C for 1 week or -20°C longer term.

Accuris offers a full line of qPCR enzymes. visit www.accuris-usa.com for details.

Technical Support

For trouble-shooting and tech support, contact us at info@accuris-usa.com or 908 769-5555.

Reaction Setup (20µl

Allow kit components to thaw and briefly vortex/centrifuge. Keep tubes on ice.

RNA/Primer Mix - Combine components in a nuclease-free microtube on ice:

RNA Template	10pg to 2.0µg Total RNA	5pg to 0.5µg mRNA
Random Hexaprimer or oligo (dT)	2.0µl	Use 3µl for >2µg RNA
PCR grade water	to 12µl final volume	

Prepare a **10x Primer Blend** in PCR Grade water Note: Briefly vortex and centrifuge. Heat RNA/primer mix to 70°C for 2 minutes then return to ice. This will help to relax secondary structure. *RTase Mix* - In a separate tube, prepare the RT Mix, according to number of rxns

5X RT Buffer	4µl	
High Capacity RT	1.0µl	Use 2µl for >2µg RNA
PCR grade water	to 8µl final volume	

Note: Be sure to prepare sufficient RT mix for your total number of reactions. For 96 reactions, 768µl is required. Calculate for a small excess to account for possible pipetting loss.

1. Add 4µl 5x RTase Buffer, 1.0 µl High Capacity RTase, Total RNA/mRNA, 2µl 10x Primer blend. Add PCR Grade Water for a total reaction volume of 20µl. Mix gently.
2. If using random hexaprimers, incubate for 10 minutes at 25°C.
3. Incubate 42°C for 60 minutes (45°C for high amounts of secondary structure).
4. Denature 70°C for 10 minutes.

The resulting cDNA can be stored and/or an aliquot can be used for qPCR using Accuris qMax qPCR products.

Package contents and reordering

The Accuris qMax First Strand cDNA Synthesis Flex Kit is available in 50 and 200 reaction packs. Kit includes high capacity reverse transcriptase (200u/µl), optimized 5X buffer, random hexaprimers (20µM) and oligo (dT) primers (20µM).

Accuris qMax First Strand cDNA Synthesis Flex Kit, sample pack, 5 reactions

Catalog number PR2110-S
Includes 10ul RT, 40µl 5X Buffer, 20µl Random Hexaprimers and 20µl Oligo (dT) Primers

Accuris qMax First Strand cDNA Synthesis Flex Kit, 50 reactions

Catalog number PR2110-50
Includes 50µl of RT, 200µl 5X buffer, 25µl Random Hexaprimers and 25µl Oligo (dT) Primers

Accuris qMax First Strand cDNA Synthesis Flex Kit, 200 reactions

Catalog number PR2110-200
Contains 4x50 reaction packs (see above)

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Accuris™ qMax First Strand cDNA Synthesis Flex Kit

- ☐ PR2110-S Sample pack
- ☐ PR2110-50 50 reactions
- ☐ PR2110-200 200 reactions

Package contains:
Reverse Transcriptase 5X RT Buffer,
Random Hexaprimers and Oligo (dT) Primers
Store at -20°C upon receipt

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