

RT² First Strand Kit

Catalog Number

C-03

Contents

Reagents for Twelve (12) First Strand cDNA Synthesis Reactions, 20 µl each

Description

The RT² First Strand Kit provides a rapid and convenient procedure for efficient first strand cDNA synthesis. The kit also contains an effective genomic DNA elimination step and a built-in External RNA Control. This all-in-one kit has been designed and optimized for real-time PCR-based gene expression analysis especially with SuperArray's RT²Profiler™ PCR Arrays and RT² Real-Time™ PCR Primer Sets. The kit includes a proprietary procedure to effectively eliminate contaminating genomic DNA from RNA samples before reverse transcription. Random hexamers and oligo-dT prime reverse transcription in an unbiased manner, and a reverse transcriptase synthesizes cDNA product with optimal yield and length. A built-in External RNA Control helps monitor reverse transcription efficiency and test for enzyme inhibitors contaminating your RNA samples when used together with RT² RNA QC PCR Array and RT²Profiler™ PCR Array. The magnesium and nucleotide concentrations and other buffer components are the most compatible with our RT² Real-Time™ SYBR Green PCR master mixes used in gene expression analysis with our RT²Profiler™ PCR Arrays and RT² Real-Time™ PCR Primer Sets. Remember to order C-03 with our other PCR reagents for optimal performance, especially to complete the RT²Profiler™ PCR Array System.

Materials Included

Please check the kit components immediately after you receive this package. SuperArray is not responsible for any missing items not reported within two (2) business days upon receipt.

RT² PCR Array First Strand Kit

Component	Tube ID
5X gDNA Elimination Buffer	GE
5X Reverse Transcription Buffer	BC3
RT Enzyme Mix	RE3
Primer and External Control Mix	P2
RNase-free H ₂ O	H ₂ O

Storage Conditions

The RT² PCR Array First Strand Kit is shipped frozen. For long-term storage, keep entire kit at -20 °C.

Shelf Life: All reagents are stable for 6 months after receipt of the kit if stored at the recommended temperature.

Related Products

RT² Profiler™ PCR Arrays (See www.superarray.com/ArrayList.php for complete listings)

High Performance RT² Real-Time™ PCR Master Mixes (Available for most real-time PCR instruments)

Pre-tested RT² Primer Sets for measuring expression of any human, mouse, or rat gene
(Search for your Primer Set at: www.superarray.com/QRTsearch.php)

Product Specification Sheet

Brief Protocol

1. Prepare a separate Genomic DNA Elimination mixture for each RNA sample:

Total RNA	25 ng to 5 μ g
GE (5X gDNA Elimination Buffer)	2 μ l
RNase-free H₂O to a final volume of ...	10 μ l

We recommend starting with 0.5 to 1.0 μ g of total RNA to maximize the positive call rate. Use a consistent amount of RNA for each sample.

2. Incubate at 42 °C for 5 min, and immediately place on ice for at least 1 minute.
3. Generate the following RT cocktail scaling up the recipe for the number of samples and viscosity pipetting errors:

BC3 (5X RT Buffer 3)	4 μ l
P2 (Primer and External Control Mix)	1 μ l
RE3 (RT Enzyme Mix 3)	2 μ l
H₂O , RNase-free	3 μ l
Final Volume	10 μ l

4. Briefly centrifuge all mixtures and the RT cocktail to the bottom of the tube.
5. Add 10 μ l of the RT Cocktail to each 10- μ l Genomic DNA Elimination Mixture for a final 20- μ l volume.
6. Incubate at 42 °C for 15 min.
7. Heat at 95 °C for 5 min to hydrolyze the RNA and to inactivate the reverse transcriptase.
8. Hold the finished reaction on ice until ready to use for real-time PCR, or place at -20 °C for longer-term storage.