SensiFAST™ cDNA Synthesis Kit

Shipping: On dry/blue ice

Catalog numbers

BIO-65053: 50 reactions

Batch No.: See vial

BIO-65054: 250 reactions

Store at –20 °C

meridian BIOSCIENCE™

Storage and stability:
SensiFAST cDNA Synthesis Kit is shipped on dry/blue ice and should be stored at -20 °C upon receipt. When stored under optimum conditions, the reagents are stable for a minimum of one year from date of purchase. Thaw, mix, and briefly centrifuge each component before use.

Unit definitions:
Reverse Transcriptase: One unit catalyzes the incorporation of 1 nmol of dTTP into acid-insoluble material in 10 minutes at 37 ºC in 50 mM Tris-HCl, pH 8.6, 40 mM KCl, 1 mM $MnSO_4$, 1 mM DTT, and 0.5 mM [3H]TTP, using 200 µM oligo$(dT)_{12\text{-}18}$-primed poly$(A)_n$ as template.
RNase Inhibitor: One unit inhibits 5 ng of RNase A by 50%.

Safety precautions:
Harmful if swallowed. Irritating to eyes, respiratory system and skin. Please refer to the material safety data sheet for information regarding hazards and safe handling practice.

Signal word: WARNING

Notes:
For research or further manufacturing use only.

Trademarks:

Description

SensiFAST cDNA Synthesis Kit provides a rapid and very sensitive method for first strand cDNA synthesis for use in real-time PCR (qPCR) studies. The 5x TransAmp™ Buffer provides highly optimized components for efficient reverse transcription, and includes a unique blend of anchored oligo dT and random hexamer primers to ensure unbiased 3' and 5' coverage for enhanced data accuracy. An extremely efficient reverse transcriptase delivers highly robust first strand synthesis and higher cDNA yields from a wide range of input RNA concentrations. SensiFAST cDNA Synthesis Kit offers enhanced sensitivity, efficiency and reproducibility for exceptional performance in subsequent qPCR experiments.

Components

Product Name	50 reactions	250 reactions
5x TransAmp Buffer	200 µL	1 mL
Reverse Transcriptase	50 µL	250 µL

SensiFAST cDNA Synthesis Mix Reaction Guidelines

Template Quality

- Intact, high-quality RNA is essential for the reverse transcription reaction
- All reagents for use with RNA must be prepared using nuclease-free, molecular biology grade water
- RiboSafe RNase Inhibitor is included in the Reverse Transcriptase mix to help reduce template degradation and increase yield of RT-qPCR product
- Low-copy-number genes may require an increase in starting material
- Use a suitable RNA extraction reagent e.g. TRIsure™ or ISOLATE II RNA Mini Kit

RNA Priming

A unique blend of random hexamer and anchored oligo dT primers in the SensiFAST cDNA Synthesis Mix provides optimal sensitivity and accuracy of first-strand cDNA synthesis. Anchored oligo dT primers anneal precisely to the junction of the poly-A tail (found on the 3' end of most eukaryotic mRNAs) and the gene of interest. This ensures that the coding 3' end of mRNAs are always represented. The reverse transcriptase can also prime from the random hexamers, to give broad coverage of all the regions of the RNA and thus a cDNA pool representative of the transcritpome. The combined benefits of both priming strategies offers enhanced data quality.

Reverse Transcription

Efficient reverse transcription can normally be achieved at 42 °C for 15 minutes, as the TransAmp Buffer contains reverse transcriptase enhancers that reduce complex RNA secondary structure. For templates that have a high degree of structure, such as viral RNA and some plant RNA, we suggest using an additional 15 minute 48 °C incubation step.

No RT Control

It is important to always include the appropriate 'no RT' or 'minus RT' control reactions in your experimental design. As the reverse transcriptase is a separate component of the SensiFAST cDNA Synthesis Kit, it is possible to include a formal cDNA synthesis control that includes all components except the reverse transcriptase.

DNase I digestion of total RNA

To eliminate any residual contaminating genomic DNA that can affect highly sensitive qPCR applications (e.g. probe-based quantification of a low abundant target), we recommend using a high quality RNase-free DNase I during or after RNA extraction protocols. DNase I removal by ethanol precipitation, or with a RNA clean-up kit e.g. ISOLATE II RNA Micro Clean-Up Kit is required before proceeding with first-strand cDNA synthesis.

SensiFAST cDNA Synthesis Kit Protocol

1. Prepare the master mix on ice.

2. Vortex solutions and centrifuge briefly before use.

Total RNA or mRNA (up to 1 µg)	*n* µL
5x TransAmp Buffer	4 µL
Reverse Transcriptase	1 µL
DNase/RNase free-water*	Up to 20 µL

* Not supplied

3. Mix gently by pipetting.

4. Set up the following program in a thermal cycler:

- 25 °C for 10 min (primer annealing)
- 42 °C for 15 min (reverse transcription)
- *Optional Step: 48 °C for 15 min (for highly-structured RNA)*
- 85 °C for 5 min (inactivation)
- 4 °C hold (or chill on ice)

5. Use up to 4 µL (1/5th volume) of cDNA synthesis reaction product in a 20 µL volume qPCR. If desired, reaction product can be diluted in 10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA prior to use.

6. Alternatively, store reaction product or diluted cDNA at 4 °C for 1 week or -20 °C for long term storage.

This protocol is intended for use as a guide only; conditions will vary from reaction to reaction and may need optimization.

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Troubleshooting

Problem	Possible Cause	Recommendation
No cDNA synthesis	RNA degraded	Analyze RNA on a denaturing gel to verify integrity. Ensure that all reagents are RNase-free.
	RNA contained an RT inhibitor	Remove inhibitors, such as SDS, EDTA, formamide and pyrophosphate, by ethanol precipitation of RNA, including a 70% ethanol wash step.
	Reaction conditions not optimal	Increase the primer annealing step from 10 min up to 15 min. Increase the reverse transcription step from 15 min up to 30 min.
	Not enough starting RNA	Increase the amount of starting RNA, this can be an important factor when amplifying low-copy genes from total RNA.
Poor specificity in qPCR	Non-specific annealing of primers to template	Increase the annealing temperature in qPCR. Check for presence of pseudogenes.
	Primer dimers	Redesign primers to prevent self-annealing. Set up reactions on ice.
	Genomic DNA contamination	Treat RNA with DNase I and re-purify. If possible, use intron-spanning primers in qPCR.
Product in 'no RT' control	RNA contaminated with genomic DNA	Treat samples with DNase I and re-purify. Use intron-spanning primers if possible.

Technical Support:

If the troubleshooting guide does not solve the difficulty you are experiencing, please contact Technical Support with details of reaction setup, cycling conditions and relevant data.

Email: mbi.tech@meridianlifescience.com

Associated products:

Product Name	Cat. No.
SensiFAST™ SYBR No-ROX Kit	BIO-98002
SensiFAST™ Probe No-ROX Kit	BIO-86005
ISOLATE II RNA Mini Kit	BIO-52072
ISOLATE II RNA Micro Kit	BIO-52075
TRIsure™	BIO-38032
HyperLadder™ 1kb	BIO-33025

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