Associated products

Product	Description	Pack Size	Cat No.
ISOLATE II Genomic DNA Kit	Rapid isolation of high-quality genomic DNA from many different starting material	10 Preps 50 Preps 250 Preps	BIO-52065 BIO-52066 BIO-52067
ISOLATE II Plant DNA Kit	Rapid isolation of high-quality genomic DNA from a wide variety of plant species	10 Preps 50 Preps 250 Preps	BIO-52068 BIO-52069 BIO-52070
ISOLATE II RNA Mini Kit	Isolation of high-yield and extremely pure total RNA from a variety of samples	10 Preps 50 Preps 250 Preps	BIO-52071 BIO-52072 BIO-52073
ISOLATE II RNA Plant Kit	Isolation of high-yield and extremely pure total RNA from a wide variety of plant species	10 Preps 50 Preps	BIO-52076 BIO-52077
TRIsure™	Quick isolation of high-quality RNA from a variety of sources for subsequent use in cDNA synthesis	100 mL 200 mL	BIO-38032 BIO-38033
SensiFAST™ cDNA Synthesis Kit	Fully optimized to generate maximum yields of full-length and low abundance cDNA from RNA	50 Reactions 250 Reactions	BIO-65053 BIO-65054
Agarose	Molecular biology grade agarose	100 g 500 g	BIO-41026 BIO-41025

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.

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Trademark and licensing information

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SensiFAST™ Probe Lo-ROX One-Step Kit

Storage and stability:
The SensiFAST Probe Lo-ROX One-Step Kit is shipped on dry/blue ice. All kit components should be stored at -20°C upon receipt. Excessive freeze/thawing is not recommended.

Expiry:
When stored under the recommended conditions and handled correctly, full activity of the kit is retained until the expiry date on the outer box label.

Quality control:
The SensiFAST Probe Lo-ROX One-Step Kit and its components are extensively tested for activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

Safety precautions:
Please refer to the material safety data sheet for further information.

Shipping: On dry/blue ice
Batch No.: See vial
Concentration: See vial

Catalog numbers
BIO-78001: 100 x 20 µL reactions: 1 x 1 mL
BIO-78005: 500 x 20 µL reactions: 5 x 1 mL

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Store at –20°C

Description

The SensiFAST™ Probe Lo-ROX One-Step Kit has been formulated for highly reproducible first-strand cDNA synthesis and subsequent real-time PCR in a single tube. The kit is formulated for use with probe-detection technology, including TaqMan®, Scorpions® and molecular beacon probes. A combination of the latest advances in buffer chemistry together with a reverse transcriptase and hot-start DNA polymerase system ensures that SensiFAST Probe Lo-ROX One-Step Kit produces fast, highly-specific and ultra-sensitive one-step real-time RT-PCR.

The SensiFAST Probe Lo-ROX One-Step Kit consists of a 2x SensiFAST Probe One-Step mix, separate reverse transcriptase and RiboSafe RNase Inhibitor.
This reagent has been manufactured under 13485 Quality Management System and is suitable for further manufacturing use as an IVD component.

Kit components

Reagent	100 x 20 µL reactions	500 x 20 µL reactions
SensiFAST™ Probe One-Step mix (2x)	1 x 1 mL	5 x 1 mL
RiboSafe RNase Inhibitor	1 x 40 µL	1 x 200 µL
Reverse transcriptase	1 x 20 µL	1 x 100 µL
DEPC-H_2O	1 x 1.8 mL	2 x 1.8 mL

Instrument compatibility

The SensiFAST Probe Lo-ROX One-Step Kit has been optimized for use with all probe chemistries, including TaqMan, FRET, Scorpions and molecular beacon probes on the real-time PCR instruments listed in the following compatibility table. Each of these instruments having the capacity to analyze the real-time PCR data with the passive reference signal either on or off. The kit is also compatible with several instruments that do not require the use of ROX, such as the Mic, (BMS), Qiagen (Corbett) Rotor-Gene™ 6000, Bio-Rad CFX96 or Roche LightCycler® 480.

Manufacturer	Model
ABI (Invitrogen)	7500, 7500 FAST
Stratagene (Agilent)	Mx4000™, Mx3000P™, Mx3005P™

General considerations

When handling RNA, it is important to use RNase-free plasticware and reagents. We also recommend performing RNA work in an RNase-free area. To help prevent any carry-over DNA contamination, we recommend that separate areas are maintained for reaction set-up, PCR amplification and any post-PCR gel analysis. It is essential that any tubes containing amplified DNA product are not opened in the PCR set-up area.

Primers and probe: These guidelines refer to the use of dual-labeled probes. Please refer to the relevant literature when using other probe types. The sequence and concentration of the probe and primers, as well as amplicon length, can be critical for specific amplification, yield and overall efficiency of any real-time RT-PCR.

We strongly recommend taking the following points into consideration when designing and running your real-time RT-PCR:

- use primer-design software, such as Primer3 or visual OMP™ (http://frodo.wi.mit.edu/primer3/ and DNA Software, Inc. http://dnasoftware.com/, respectively). Primers should have a melting temperature (Tm) of approximately 60°C. The Tm of the probe should be approximately 10°C higher than that of the primers
- optimal amplicon length should be 80-200 bp, and should not exceed 400 bp
- final primer concentration of 400 nM is suitable for most probe reactions. However, to determine the optimal concentration we recommend titrating in the range 0.2-1 µM
- use an equimolar primer concentration
- a final probe concentration of 100nM is suitable for most applications. We recommend that the final probe concentration is at least 2-fold lower than the primer concentration
  Note: In multiplex real-time RT-PCR, probe concentrations in excess of 100nM can result in cross-channel fluorescence
- where possible, use intron-spanning primers to avoid amplification from genomic DNA

Template: It is important that the RNA template is intact and devoid of DNA or contaminating inhibitors of both reverse transcription and PCR. For high purity RNA, we recommend using the Bioline ISOLATE II RNA Mini Kit (BIO-52073). RNA stocks and dilutions should be made in DEPC-treated water to avoid any RNase-mediated degradation.

The recommended amount of template for one-step real-time RT-PCR is dependent upon the type of RNA used:

- **total RNA:** purified total RNA can be used in the range from 1 pg to 1 µg per 20 µL reaction.
- **mRNA:** purified mRNA can be used from 0.01 pg per 20 µL reaction.

$MgCl_2$: The $MgCl_2$ concentration in the 1x reaction mix is 3 mM. In the majority of real-time RT-PCR conditions this is optimal for both the reverse transcriptase and the hot-start DNA polymerase. If necessary, we suggest titrating the $MgCl_2$ to a maximum of 5 mM.

RT-PCR controls: It is important to detect the presence of contaminating DNA that may affect the reliability of the data. Always include a no-RT control reaction, by omitting the reverse transcriptase from the reaction.

Optional ROX: The SensiFAST Probe Lo-ROX One-Step Kit is premixed with ROX (5-carboxy-X-rhodamine, single isomer), so that ROX fluorescence can be optionally detected on certain real-time instruments. If your real-time PCR instrument has the capability of using ROX and you wish to use this option, then this option must be selected by the user in the software.

Procedure

Reaction mix composition: Prepare an real-time RT-PCR mastermix. The volumes given below are based on a standard 20µl final reaction mix and can be scaled accordingly.

Reagent	Volume	Final concentration
2x SensiFAST Probe Lo-ROX One-Step Mix	10 µL	1x
10 µM Forward Primer	0.8 µL	400 nM
10 µM Reverse Primer	0.8 µL	400 nM
10 µM Probe	0.2 µL	100 nM
Reverse transcriptase	0.2 µL	-
RiboSafe RNase Inhibitor	0.4 µL	-
H_2O	up to 16 µL	
Template	4 µL	
	20 µL Final volume	

Sensitivity testing and C_t values: When comparing SensiFAST with a mix from another supplier we strongly recommend amplifying from a 10-fold template dilution series. Loss of detection at low template concentration is the only direct measurement of sensitivity. An early C_t value is not an indication of good sensitivity, but rather an indication of speed.

Suggested real-time RT-PCR conditions: The following real-time RT-PCR conditions are suitable for the SensiFAST Probe Lo-ROX One-Step Kit with the majority of amplicons and real-time PCR instruments. However, the cycling conditions can be varied to suit different probe-based reactions or machine-specific protocols. The detection channel on the real-time instrument should be set to acquire at the appropriate wavelength(s). We recommend using the following cycling conditions for optimal results:

- **Cycling for dual-labeled probes**

Cycles	Temp.	Time	Notes
1	45°C	10 min	Reverse transcription
1	95°C	2 min	Polymerase activation
40	95°C 60°C	5s 20s	Denaturation Annealing/extension (acquire at end of step)

Real-Time RT-PCR optimization: The following optimization may be necessary to improve the efficiency of some reactions, such as multiplexing with more than two probes, or if the target amplicon is longer than 200 bp.

- The reverse transcription reaction time can be extended up to 20 minutes and/or the temperature can be increased up to 48°C
- The annealing/extension time can be extended up to 60 seconds and/or the temperature can be increased up to 65°C

Troubleshooting guide

Problem	Possible Cause	Recommendation
No amplification trace AND No product on agarose gel	Activation time too short	Ensure SensiFAST Probe Lo-ROX One-Step mix is activated for a minimum of 2 min at 95°C before cycling
	Error in protocol setup	Verify that correct reagent concentrations, volumes, dilutions and storage conditions have been used
	Suboptimal primer/probe design	Use primer design software or validated primers/probes. Test assay on a control template
	Incorrect concentration of primers/probe	Use primer concentrations between 200 nM and 1 µM. Probe concentration should be at least 2-fold lower than the primer concentration
	Template degraded	Re-isolate your template from the sample material or use freshly prepared template dilution. We recommend using the ISOLATE RNA kits for template preparation and DEPC-treated water for resuspension or dilution of the template Verify the integrity of RNA using agarose gel electrophoresis
	Primers/probe degraded	Use newly synthesized primers and/or probe
	Template contaminated with real-time RT-PCR inhibitors	Further dilute template before real-time RT-PCR or purify template and resuspend it in DEPC-treated water
	Template concentration too low	Increase concentration used
	Cycling conditions not optimal	Increase extension/annealing time, increase cycle number, reduce annealing temperature

Troubleshooting guide (Continued)

Problem	Possible Cause	Recommendation
No amplification trace AND PCR product present on agarose gel	Error in instrument setup	Check that the acquisition settings are correct during cycling
Non-specific amplification product AND Primer-dimers	Inefficient reverse transcription	Extend reverse transcription time up to 20 min and/or increase the temperature up to 48°C
	Suboptimal primer/probe design	Redesign primers and/or probe using appropriate software, or use validated primers/probes
	Primer/probe concentration too high	Test dilution series of primer/probe concentrations until primer-dimer/non-specific amplification products disappear
	Primer/probe concentration too low	Use primer concentration between 200 nM and 1 µM and probe concentration at least 2 fold lower
	Annealing/extension temperature too low	Increase annealing/extension temperature up to 65°C or until primer-dimer/non-specific amplification products disappear
	Template concentration too low	Increase template concentration
	Template concentration too high	Reduce template concentration until non-specific products disappear
	Extension time too long	Reduce extension time to determine whether non-specific products are reduced
Variability between replicates	Error in reaction set-up	Prepare large volume mastermix
	Air bubbles in reaction mix	Centrifuge reaction samples/plate prior to running on a real-time instrument
Late amplification trace	Inefficient reverse transcription	Extend reverse transcription time up to 20 min and/or increase the temperature up to 48°C
	Activation time too short	Ensure SensiFAST Probe Lo-ROX One-Step mix is activated for a minimum of 1 min at 95°C before cycling
	Annealing temperature too high	Decrease annealing temperature in steps of 2°C
	Extension time too short	Double extension time to determine whether the cycle threshold (C_t) is affected
	Template concentration too low	Increase concentration if possible
	Template is degraded	Re-isolate template from sample material or use freshly prepared template dilution
	Suboptimal primer/probe design	Redesign primers/probe using appropriate software, or use validated primers
	Primer/probe concentration too low	Increase concentration of primers in 100 nM increments and probe concentration in increments at least 2 fold lower than that of the primer
	RNase contamination	Ensure RNase inhibitor is added before addition of template
PCR efficiency below 90%	Extension time too short	Increase extension time
	Primer concentration too low	Increase concentration of primers in 100nM increments
	Suboptimal primer/probe design	Redesign primer/probe using appropriate software or use validated primer/probe
PCR efficiency above 110%	Template is degraded or contains PCR inhibitors	Re-isolate template from sample material, or use freshly prepared template dilution, or purify template and resuspend it in water
	Non specific amplification and/or primer-dimers	Use 4% agarose gel electrophoresis to confirm presence of non-specific amplification products. See above for preventing/removing non-specific products