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
SensiFAST™ Probe Direct SuperMix	Highly accurate, reproducible assay results in the presence of inhibitors, with DNA or cDNA	500 Reactions 2000 Reactions	BIO-86105 BIO-86120
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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Storage and stability:
SensiFAST Probe One-Step Direct Lo-ROX SuperMix 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:
SensiFAST Probe One-Step Direct Lo-ROX SuperMix 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.

Notes:
For research or further manufacturing use only.

Trademarks:
SensiFAST (Bioline Reagents Ltd), ROX (Roche), StepOne (ABI), Mx4000, Mx3000P and Mx3005P (Stratagene), iCycler, MyiQ5, Opticon, Chromo4, Miniopticon, (Bio-Rad), Light-Cycler (Roche), SmartCycler (CEPheid), RotorGene (Qiagen), RealPlex (Eppendorf), Quantica (Techne). TaqMan (ABI).

SensiFAST™ Probe One-Step Direct Lo-ROX SuperMix

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

Catalog numbers
BIO-74101: 200 x 20 µL reactions: 1 x 1 mL
BIO-74105: 1000 x 20 µL reactions: 5 x 1 mL

Store at –20°C

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Description

SensiFAST™ Probe One-Step Direct Lo-ROX SuperMix is a 4x mix that has been designed for highly accurate, reproducible assay results in the presence of inhibitors, making it ideal for amplification directly from human and animal blood samples and, more generally biofluids containing PCR inhibitors. The kit has been formulated for use with probe-detection technology, such as TaqMan®. A combination of the latest advances in buffer chemistry and PCR enhancers, together with a hot-start DNA polymerase and reverse transcriptase, ensures that the SensiFAST Probe One-Step Direct Lo-ROX SuperMix delivers fast, highly-specific and ultra-sensitive RT-qPCR.

Kit components

Reagent	200 x 20 µL reactions	1,000 x 20 µL reactions
SensiFAST™ Probe One-Step Direct Lo-ROX SuperMix (4x)	1 x 1 mL	5 x 1 mL

Instrument compatibility

SensiFAST Probe One-Step Direct Lo-ROX SuperMix has been optimized for use with all probe chemistries, including TaqMan, FRET, Scorpions and molecular beacon probes on qPCR instruments listed in the following compatibility table, each of these instruments having the capacity to analyze the qPCR 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 Rotor-Gene™ 6000, the Bio-Rad CFX96 or the Roche LightCycler® 480.

Manufacturer	Model
ABI (Invitrogen)	7500, 7500 FAST, ViiA7
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 reaction 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 RT-qPCR.

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

- use primer-design software, such as Primer3 (http://frodo.wi.mit.edu/primer3/) or visual OMP™ (http://dnasoftware.com/). 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 100 nM 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 RT-qPCR, probe concentrations in excess of 100 nM can result in cross-channel fluorescence
- where possible, use intron-spanning primers to avoid amplification from genomic DNA

Template: SensiFAST Probe One-Step Direct Lo-ROX SuperMix can be used for amplification from crude lysates or inhibitor-rich samples such as urine, cerebral spinal fluid (CSF) or blood. Due to the high viscosity, pipette a minimum of 4 µL of undiluted or diluted blood. The maximum recommended final concentration of blood in the reaction will be dependent of the qPCR platform, this may vary between 5 and 20 %. Some optimization may be required.

SensiFAST Probe One-Step Direct Lo-ROX SuperMix can also be used on purified RNA template to improve reproducibility from contaminating inhibitors that have come through the purification process The recommended amount of template for one-step RT-qPCR 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 SensiFAST Probe One-Step Direct Lo-ROX SuperMix contains an optimized concentration of $MgCl_2$, it is not necessary to supplement the mix further.

Optional ROX: SensiFAST Probe One-Step Direct Lo-ROX SuperMix is premixed with ROX (5-carboxy-X-rhodamine, succinymidyl ester), so that where necessary, ROX fluorescence can be optionally detected on certain real-time instruments. If your real-time 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 RT-qPCR master mix. The volumes given below are based on a standard 20 µL final reaction mix and can be scaled accordingly.

Reagent	Volume	Final concentration
4x SensiFAST Probe One-Step Direct Lo-ROX SuperMix	5 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
H_2O	As required	
Template	As required	
20 µL Final volume		

Suggested RT-qPCR conditions: The following RT-qPCR conditions are suitable for the SensiFAST Probe One-Step Direct Lo-ROX SuperMix 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:

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.

- **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	5 s 20 s	Denaturation Annealing/extension (acquire at end of step)

RT-qPCR 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 One-Step Direct Lo-ROX SuperMix 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
	Primers/probe degraded	Use newly synthesized primers and/or probe
	Template contaminated with RT-qPCR inhibitors	Further dilute template before RT-qPCR 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
No amplification trace AND PCR product present on agarose gel	Error in instrument setup	Check that the acquisition settings are correct during cycling
Variability between replicates	Error in reaction set-up	Prepare large volume master mix
	Air bubbles in reaction mix	Centrifuge reaction samples/plate prior to running on a real-time instrument

Troubleshooting guide (Continued)

Problem	Possible Cause	Recommendation
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
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 One-Step Direct Lo-ROX SuperMix is activated for a minimum of 1min 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
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