## Associated products

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

Email: tech@bioline.com

## Trademark and licensing information

1).Trademarks: SensiMix™ (Bioline Reagents Ltd), SYBR® (Molecular Probes), iCycler™ MyiQ5™, Opticon™, Chromo4™, Miniopticon™, (Bio-Rad), LightCycler® (Roche), StepOne™ (ABI), SmartCycler™ (CEPheid), RotorGene™ (Corbett), RealPlex™ (Eppendorf), Quantica™ (Techne), MX4000 (Stratagene) Eco™ (PCRmax), Thermal Cycler Dice® (Takara)

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**Storage and Stability:**
The SensiFAST Probe No-ROX 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 No-ROX 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.

# SensiFAST™ Probe No-ROX Kit

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

Catalog numbers
BIO-86002: 200 x 20 µL reactions: 2 x 1 mL
BIO-86005: 500 x 20 µL reactions: 5 x 1 mL
BIO-86020: 2000 x 20 µL reactions: 4 x 5 mL

BIOLINE
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**Store at –20°C**

## Description

The SensiFAST™ Probe No-ROX Kit has been developed for fast, highly reproducible real-time PCR and has been validated on commonly used real-time PCR instruments. The kit has been formulated for use with probe-detection technology, including TaqMan®, Scorpions® and molecular beacon probes. A combination of the latest advances in buffer chemistry and PCR enhancers, together with a hot-start DNA polymerase, ensures that the SensiFAST Probe Kit delivers fast, highly-specific and ultra-sensitive real-time PCR.

SensiFAST Probe is provided as a 2x mastermix containing all the components necessary for real-time PCR, including dNTPs, stabilizers and enhancers.

## Kit components

| Reagent | 200 x 20 µL Reactions | 500 x 20 µL Reactions | 2000 x 20 µL Reactions |
|---|---|---|---|
| SensiFAST Probe No-ROX mix (2x) | 2 x 1 mL | 5 x 1 mL | 4 x 5 mL |

## Instrument compatibility

The SensiFAST Probe No-ROX Kit is compatible with real-time PCR instruments that do not need a passive reference signal for normalization of the data. The SensiFAST Probe No-ROX Kit has been optimized for use on the real-time PCR instruments listed in the following compatibility table.

| Manufacturer | Model |
|---|---|
| Bio-Rad | iCycler®, iQ™5, MyiQ™, Opticon™, Opticon2™, MiniOpticon, Chromo4™, CFX96, CFX384 |
| Cepheid | SmartCycler™ |
| Qiagen | Rotor-Gene™ 3000 & 6000 |
| Eppendorf | Mastercycler® ep realplex |
| Roche | LightCycler® 480 |
| Techne | Quantica® |
| BMS | Mic |
| Takara | Thermal Cycler Dice® (TP800) |

## General considerations

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 PCR product are not opened in the PCR set-up area.

**Primers and probe:** These guidelines refer to the design and set-up of TaqMan probe-based PCR. Please refer to the relevant literature when using other probe types. The specific amplification, yield and overall efficiency of any real-time PCR can be critically affected by the sequence and concentration of the probes and primers, as well as by the amplicon length.

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

- 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 300 bp
- final primer concentration of 400 nM is suitable for most Probe-based reactions, however to determine the optimal concentration we recommend titrating in the range 0.2-1 µM. The forward and reverse primers concentration should be equimolar
- a final probe concentration of 100 nM is suitable for most applications; we recommend that the final probe concentration is at least two-fold lower than the primer concentration
  *Note: Multiplex real-time PCR probe concentrations in excess of 100nM, can result in cross-channel fluorescence*

**Template:** It is important that the DNA template is suitable for use in PCR in terms of purity and concentration. In addition, the template must be devoid of any contaminating PCR inhibitors (e.g. EDTA). The recommended amount of template for PCR is dependent upon the type of DNA used. The following points should be considered when using genomic DNA and cDNA templates:

- **Genomic DNA:** use up to 1 µg of complex (e.g. eukaryotic) genomic DNA in a single PCR; we recommend using the Bioline ISOLATE II Genomic DNA Kit (BIO-52066) for high yield and purity from both prokaryotic and eukaryotic sources.
- **cDNA:** the optimal amount of cDNA to use in a single PCR is dependent upon the copy number of the target gene; we suggest using 100 ng cDNA per reaction, however it may be necessary to vary this amount; to perform a two-step RT-PCR, we recommend using the SensiFAST cDNA Synthesis Kit (BIO-65053) for reverse transcription of the purified RNA; for high yield and purity of RNA, use Bioline ISOLATE II RNA Mini Kit (BIO-52072).

**$MgCl_2$:** The SensiFAST Probe mix contains an optimized concentration of $MgCl_2$, it is not necessary to supplement the mix further.

**PCR controls:** It is important to detect the presence of contaminating DNA that may affect the reliability of the data. Always include a no-template control (NTC) reaction, replacing the template with PCR-grade water. When performing a two-step RT-PCR, set up a no-RT control as well as an NTC for the PCR.

## Procedure

**Reaction mix composition:** Prepare a PCR mastermix. The volumes given below are based on a standard 20 µL final reaction mix and can be scaled accordingly.

| Reagent | Volume | Final concentration |
|---|---|---|
| 2x SensiFAST Probe No-ROX 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 |
| Template | up to 8.2 µL | |
| $H_2O$ | As required | |

**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 thermal cycling conditions

The real-time PCR conditions, in the table below, are suitable for the SensiFAST Probe No-ROX Kit with the amplicons of up to 200 bp. These cycling parameters have been optimized on a number of platforms, however they can be varied to suit different machine-specific protocols.

| Cycles | Temp. | Time | Notes |
|---|---|---|---|
| 1 | 95°C | *2-5min | Polymerase activation |
| 40 | 95°C | 10s | Denaturation |
| | 60°C | **20-50s | Annealing/extension (acquire at end of step) |

*2 min for cDNA, up to 5min for genomic DNA
**Up to 50s may be necessary for multiplexing with more than two probes

## Troubleshooting guide

| Problem | Possible Cause | Recommendation |
|---|---|---|
| No amplification trace AND No product on agarose gel | Activation time too short | For cDNA templates, make sure SensiFAST Probe No-ROX is activated for 2 min at 95°C before cycling. For more complex templates such as genomic DNA, increase activation time up to 5 minutes |
| | Error in protocol setup | Verify that correct reagent concentrations, volumes, dilutions and storage conditions have been used |
| | Suboptimal primer design | Use primer/probe design software or validated primers. Test primers on a control template |
| | Incorrect concentration of primers/probe | Use primer concentration between 300 nM and 1 µM and probe concentration of 100 nM |
| | Template degraded | Re-isolate your template from the sample material or use freshly prepared template dilution |
| | Primers/probe degraded | Use newly synthesized primers and probe |
| | Template contaminated with PCR inhibitors | Further dilute template before PCR or purify template and resuspend it in PCR-grade water |
| | Template concentration too low | Increase concentration used |
| | Cycling conditions not optimal | Increase extension/annealing times, increase cycle number |
| No amplification trace AND PCR product present on agarose gel | Error in instrument setup | Check that the acquisition settings are correct during cycling |



## Troubleshooting guide (Continued)

| Problem | Possible Cause | Recommendation |
|---|---|---|
| Non-specific amplification product AND Primer-dimers | Suboptimal primer/probe design | Use primer/probe design software or validated assays. Test primer/probe on a control template |
| | Primer/probe concentration too high | Test dilution series of primer concentrations until primer dimer/non-specific amplification products disappear |
| | Primer/probe concentration too low | Use primer concentration between 300 nM and 1 µM and probe concentration of 100 nM |
| | Primer annealing/extension temperature(s) too low | Due to the high ionic strength of SensiFAST Probe No-ROX Kit it is not recommended to use annealing/extension temperatures below 60°C. Annealing/extension temperature can be increased in steps of 2°C in the event of non-specific products |
| | 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, vortex thoroughly and aliquot into reaction plate |
| | Air bubbles in reaction mix | Centrifuge reaction samples/plate prior to running on a real-time instrument |
| Late amplification trace | Activation time too short | Ensure the reaction is activated for between 2 min and 5min at 95°C before cycling |
| | Extension time too short | Increasing the extension time may be necessary for amplification products over 200bp; double extension time to determine whether the cycle threshold ($C_t$) is affected |
| | Annealing temperature too high | Decrease annealing temperature in steps of 2°C |
| | Template concentration too low | Increase concentration if possible |
| | Template is degraded | Re-isolate template from sample material or use freshly prepared template dilution |
| | Suboptimal design of primers | Redesign primers using appropriate software or use validated primers |
| | Primer/probe concentration too low | Use primer concentration between 300 nM and 1 µM and probe concentration of 100 nM |
| PCR efficiency below 90% | Extension time is too short | Increase extension time |
| | Primer/probe concentration too low | Use primer concentration between 300 nM and 1 µM and probe concentration of 100 nM |
| | Suboptimal design of primers/ probe | Use primer/probe design software or validated assays. Test primer/probe on a control template |