

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 |

TRADEMARK AND LICENSING INFORMATION

1). Trademarks: SensiMix™ and SensiFAST™ (Bioline Reagents Ltd), SYBR® (Molecular Probes), ROX™, iCycler™ MyiQ5™, Opticon™, Chromo4™, MiniOpticon™, (Bio-Rad), LightCycler® (Roche), StepOne™ (ABI), SmartCycler™ (CEPheid), RotorGene™ (Corbett), RealPlex™ (Eppendorf), Quanta™ (Techne), MX4000 (Stratagene).

2). Purchase of this product conveys a licence from Life Technologies to use this SYBR® containing reagent in an end-user RUO assay. Parties wishing to incorporate this SYBR® containing reagent into a downstream kit, should contact Life Technologies for SYBR® Licencing information.

| | | | | | |
|--|--|--|--|--|---|
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Website: www.bioline.com/sensimix email: info@bioline.com

Storage and Stability:

SensiMix SYBR® 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:

SensiMix SYBR® 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 prior to release.

Safety Precautions:

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

Notes:

Research Use Only

Description

The SensiMix™ SYBR® No-ROX Kit is a high-performance reagent designed for superior sensitivity and specificity on various real-time instruments, in which a passive reference signal is not required. The SensiMix SYBR® No-ROX Kit employs a hot-start DNA polymerase, for high PCR specificity and sensitivity. SensiMix is inactivated and possesses no polymerase activity during the reaction set-up, preventing non-specific amplification including primer-dimer formation.

For ease-of-use and added convenience, SensiMix SYBR® No-ROX is provided as a 2x mastermix containing all the components necessary for real-time PCR, including the SYBR® Green I dye, dNTPs, stabilisers and enhancers. As a ready-to-use premix, only primers and template need to be added.

Kit components

| Reagent | 250 x 50 µL reactions | 500 x 50 µL reactions | 2000 x 50 µL reactions |
|-----------------------------|-----------------------|------------------------|------------------------|
| SensiMix™ SYBR® No-ROX (2x) | 5 x 1.25 mL (6.25 mL) | 10 x 1.25 mL (12.5 mL) | 40 x 1.25 mL (50 mL) |
| 50 mM MgCl ₂ | 1 x 1 mL | 1 x 1 mL | 4 x 1 mL |

Kit compatibility

The SensiMix SYBR® No-ROX Kit contains premixed SYBR® Green I dye for compatibility with real-time instruments that do not need a passive reference signal for normalization of the data. The SensiMix SYBR® No-ROX Kit is optimized for use on the real-time instruments listed in the following compatibility table.

| Manufacturer | Model |
|--------------|---|
| Bio-Rad | Opticon™, Opticon2™, MiniOpticon, Chromo4™, CFX96, CFX384 |
| Cepheid | SmartCycler™ |
| Qiagen | Rotor-Gene™ 3000 & 6000 |
| Eppendorf | Mastercycler ep Realplex, ep Reaplex 2S |
| Roche | LightCycler® 480 |
| Techne | Quanta® |
| BMS | Mic |
| Takara | Thermal Cycler Dice® TP800 |

General considerations

To help prevent any carry-over DNA contamination we recommend that separate areas be maintained for PCR set-up, PCR amplification and any post-PCR gel analysis. It is essential that any amplified PCR product should not be opened in the PCR set-up area.

SensiMix™ SYBR® No-ROX Kit

Shipping: On Dry/Blue Ice Catalog Numbers

Batch No.: See vial QT650-02: 250 x 50 µL reactions: 5 x 1.25 mL

Concentration: See vial QT650-05: 500 x 50 µL reactions: 10 x 1.25 mL

QT650-20: 2000 x 50 µL reactions: 40 x 1.25 mL



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Store at -20 °C

Primers: the sequence and concentration of primer as well as the amplicon length can be critical for specific amplification, yield and overall efficiency of any real-time PCR. We strongly recommend taking the following into consideration when designing and running your PCR reaction:

- 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 (T_m) of approximately 60 °C

- optimal amplicon length should be 50-150 bp

- a final primer concentration of 250 nM is suitable for most PCR conditions, however to determine the optimal concentration we recommend a primer titration in the range of 0.1–1 µM

- use equimolar primer concentrations

- when amplifying from cDNA use gene-specific primers. If possible use intron-spanning primers to avoid amplification from genomic DNA

Template: it is important that the DNA template is suitable for use in PCR in terms of purity and concentration. Also, the template needs to 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 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 Mini 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 the Bioline ISOLATE II RNA Mini Kit (BIO-52072)

MgCl₂: The MgCl₂ concentration in the 1x reaction mix is 3 mM, which is optimal for SensiMix in the majority of real-time PCR conditions. If necessary, we suggest titrating MgCl₂ to a maximum of 5 mM.

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

Procedure

Reaction mix composition: Prepare a PCR master mix. The volumes given below are based on a standard 50µl final reaction mix and can be scaled accordingly.

| Reagent | Volume | Final concentration |
|---------------------------|-------------|---------------------|
| 2x SensiMix™ SYBR® No-ROX | 25 µL | 1x |
| 25 µM Forward Primer | 0.5 µL | 250 nM |
| 25 µM Reverse Primer | 0.5 µL | 250 nM |
| H ₂ O | Up to 45 µL | - |
| Template | 5 µL | |
| 50 µL Final volume | | |

Suggested thermal cycling conditions

The PCR conditions described below are suitable for SensiMix SYBR® No-ROX Kit for the majority of amplicons and real-time PCR instruments. However, the cycling conditions can be varied to suit customer or machine-specific protocols. The critical step of the PCR is the 10 minute initial activation at 95 °C. The detection channel on the real-time instrument should be set to (SYBR®) Green or FAM.

| Cycles | Temperature | Time | Notes |
|--------|----------------------------|----------------------|--|
| 1 | *95 °C | *10 min | Polymerase activation |
| 40 | 95 °C 55-60 °C 72 °C | 15 s 15 s 15 s | Temp. depends on the T _m of primers Acquire at end of step |

***Non-variable parameter**

Optional analysis:

After the reaction has reached completion refer to the instrument instructions for the option of melt-profile analysis.

Troubleshooting Guide

| Problem | Possible Cause | Recommendation |
|--|---|--|
| No amplification trace AND No product on agarose gel | Activation time too short | Make sure SensiMix is activated for 10min at 95 °C before cycling |
| | Error in protocol setup | Verify that correct reagent concentrations, volumes, dilutions and storage conditions have been used |
| | Suboptimal primer design | Use primer design software or validated primers. Test primers on a control template |
| | Incorrect concentration of primers | Use primer concentration between 100 nM and 1 µM |
| | Template degraded | Re-isolate your template from the sample material or use freshly prepared template dilution |
| | Primers degraded | Use newly synthesized primers |
| | Template contaminated with PCR inhibitors | Further dilute template before PCR or purify template and resuspend it in PCR-grade H ₂ O |
| | Template concentration too low | Increase concentration used |
| Cycling conditions not optimal | Increase extension/annealing times, increase cycle number, reduce annealing temperature | |
| No amplification trace AND Product 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 design | Redesign primers using appropriate software or use validated primers |
| | Primer concentration too high | Test dilution series of primer concentrations until primer dimer/non-specific amplification products disappear |
| | Primer concentration too low | Titrate primers in the concentration range of 100nM - 1 µM |
| | Primer annealing temperature too low | Increase PCR annealing temperature in increments of 2 °C 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 |
| Late amplification trace | Extension time too long | Reduce extension time to determine whether non-specific products are reduced |
| | Activation time too short | Ensure the reaction is activated for 10 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 with high secondary structure | Increase reverse transcription reaction time up to 30 min Increase reverse transcription reaction temperature up to 45 °C |
| | Template is degraded | Re-isolate template from sample material or use freshly prepared template dilution |
| PCR efficiency below 90% | Suboptimal design of primers | Redesign primers using appropriate software or use validated primers |
| | Primer concentration too low | Increase concentration of primer in 100 nM increments |
| | Extension time is too short | Increase extension time |
| 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 H ₂ O |
| | Non specific amplification and/or primer dimers | Use melt analysis and 4% agarose gel electrophoresis to confirm presence of non-specific amplification products. See above for preventing/removing non-specific products |

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