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 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

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

Bioline Reagents Ltd
UNITED KINGDOM
Tel: +44 (0)20 8830 5300
Fax: +44 (0)20 8452 2822

Bioline USA Inc.
USA
Tel: +1 508 880 8990
Fax: +1 508 880 8993

Bioline GmbH
GERMANY
Tel: +49 (0)337 168 1229
Fax: +49 (0)3371 68 1244

Bioline (Aust) Pty. Ltd
AUSTRALIA
Tel: +61 (0)2 9209 4180
Fax: +61 (0)2 9209 4763

Bioline France
FRANCE
Tel: +33 (0)1 42 56 04 40
Fax: +49 (0)9 70 06 62 10

Meridian Bioscience Asia Pte Ltd
SINGAPORE
Tel: +65 6774 7196
Fax: +65 6774 6441

Storage and stability:
The SensiFAST Hi-ROX Genotyping 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 Hi-ROX Genotyping 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™ Hi-ROX Genotyping Kit

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

Catalog numbers
BIO-35002: 200 x 20 µL reactions: 2 x 1 mL
BIO-35005: 500 x 20 µL reactions: 5 x 1 mL
BIO-35020: 2000 x 20 µL reactions: 20 x 1 mL

BIOLINE
A Meridian Life Science® Company

Store at –20°C

Description

SensiFAST™ Hi-ROX Genotyping Kit from Bioline has been developed for fast, precise and highly reproducible genotyping of sequence variants, including loci with type IV SNPs. The SensiFAST Hi-ROX Genotyping Kit is a combination of the latest advances in buffer chemistry, together with an antibody-mediated hot-start DNA polymerase system. This ensures that the SensiFAST Hi-ROX Genotyping Kit produces highly-specific, ultra-sensitive real-time PCR with clear allelic discrimination and outstanding allele clustering. The SensiFAST Hi-ROX Genotyping Kit is compatible with many dual-labeled probe assays (including TaqMan probe-based assays) and has been validated on commonly used real-time instruments.

Kit components

Reagent	200 x 20 µL reactions	500 x 20 µL reactions	2000 x 20 µL reactions
SensiFAST Hi-ROX Genotyping Mix (2x)	2 x 1 mL	5 x 1 mL	20 x 1 mL

Kit compatibility

The SensiFAST Hi-ROX Genotyping Kit can be used on several types of real-time PCR instruments. When used on ABI 7000, 7300, 7700, 7900, 7900HT, 7900HT FAST, StepOne™ and StepOne™ Plus, the user has the choice of analyzing the real-time PCR data with the passive reference signal either on or off. 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.

The SensiFAST Hi-ROX Genotyping Kit can also be used on instruments that do not require the use of ROX (5-carboxy-X-rhodamine, single isomer), such as the BMS Mic, BioRad® Opticon™, Opticon2™, MiniOpticon, Chromo4™, CFX96, CFX384, iQ5™, Cepheid® SmartCycler™, Qiagen (Corbett) Rotor-Gene™ 3000, 6000 & Q, Analytik Jena qTower2, Eppendorf Mastercycler ep Realplex, ep Realplex 2S, Roche LightCycler® 480, LightCycler® Nano, Techne Quantica®, PrimeQ, PCRmax Eco™, Takara Thermal Cycler Dice® TP800.

General considerations

To help prevent any carryover 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.

Primer and probes: The following information relates to the design and set-up of TaqMan probe-based PCR. When using other probe types, please refer to the appropriate literature.

The length, sequence and concentration of both primers and probes are critical for specific amplification. We strongly recommend taking the following points into consideration when designing and running your real-time PCR:

- 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
- A final primer concentration of 900 nM is suitable for most reactions, however to determine the optimal concentration we suggest titrating in the range of 0.2-1 µM; the forward and reverse primer concentrations should be equimolar
- A final concentration of 200 nM for each probe is sufficient for most reactions; we recommend the final probe concentrations are at least two-fold lower than the primer concentration; the concentration of both probes should be equimolar

The SensiFAST Hi-ROX Genotyping Kit is compatible with ABI TaqMan Pre-Designed SNP Genotyping assays.

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 SensiFAST Hi-ROX Genotyping Kit can be used with sample lysates or purified genomic DNA. Use 1-20 ng of genomic DNA in the reaction. It is important that all the wells used for the assay contain approximately the same amount of DNA for accurate genotype calling. We recommend using the Bioline ISOLATE II Genomic DNA Kit (BIO-52066) for high yield and purity from prokaryotic or eukaryotic sources.

$MgCl_2$: The $MgCl_2$ concentration in the 1x reaction mix is 3 mM. In the majority of real-time PCR conditions this is optimal for the hot-start DNA polymerase.

PCR controls: Always include a no-template control reaction by replacing the template with PCR-grade water. To ensure distinct genotype calling, positive controls containing genomic DNA samples of known genotype should be run with each assay.

Procedure

Reaction mix composition: The final volumes shown are based on a standard 20 µL final reaction mix and can be scaled accordingly.

Reagent	Volume	Final concentration
2x SensiFAST Hi-ROX Genotyping Mix	10 µL	1x
18 µM forward primer	1 µL	0.9 µM
18 µM reverse primer	1 µL	0.9 µM
4 µM allele1 probe	1 µL	0.2 µM
4 µM allele2 probe	1 µL	0.2 µM
Template	4 µL	1-20 ng per reaction
H_2O	Up to 20 µL	

Suggested real-time PCR conditions: The following real-time PCR conditions are suitable for the SensiFAST Hi-ROX Genotyping Kit with amplicons of up to 200 bp. However, the cycling conditions can be varied to suit machine-specific protocols. It is not recommended to use annealing temperatures below 60 °C.

- **2-step cycling**

Cycles	Temp.	Time	Notes
1	95 °C	3 min	Polymerase activation
30-45	95 °C 60 °C	10 s 30-45 s	Denaturation Annealing/extension (acquire at end of step)

We recommend running 30 cycles with a 30 s extension and adding cycles in increments of five, if required. For low concentrations of template (<1 ng) up to 45 cycles may be necessary, however it is not recommended to exceed a total of 45 cycles for optimal calling. Longer extension times may be required for amplicons larger than 200 bp. Three-step cycling may give improved performance with some primer sets.

Troubleshooting guide

Problem	Possible Cause	Recommendation
No or low amplification trace resulting in single indistinguishable cluster in scatter plot	Error in protocol setup	Verify that the 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. Ensure no SNP is present in the primer region.
	Incorrect concentration of primers	Use a final primer concentration that is at least twice that of the probe.
	Template degraded	Re-isolate your template from sample material or use freshly prepared dilutions.
	Primers degraded	Use newly synthesized primers.
	Template contaminated with PCR inhibitors	Further dilute template before PCR or re-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.
Fluorescent signal too low for genotype discrimination	Too few cycles	Return the plate to the thermal cycler and run for a further five cycles.

Troubleshooting guide (Continued)

Problem	Possible Cause	Recommendation
No clusters in scatter plot	Template contaminated with PCR inhibitors	Further dilute template before PCR or purify template and resuspend it in PCR-grade water.
	Template degraded	Re-isolate your template from sample material or use freshly prepared dilutions.
	Wrong reporter dye selected	Correct dye settings on your real-time PCR instrument.
More than three clusters in scatter plot	More than one SNP	Check the SNP database (http://www.ncbi.nlm.nih.gov/snp) for the presence of additional or newly discovered SNPs.
	More than one copy of SNP or SNP is multi-allelic	Sequence the DNA samples and compare.
	Sample contamination	Test the sample integrity by running the samples on alternative assays.
Clusters appear "stretched" in scatter plot	Variable sample concentration	Run samples on agarose gel to check for degradation. Check DNA concentrations and ensure comparable amounts are used for each sample.
	PCR inhibitors	Dilute the DNA sample or re-purify template and resuspend in PCR-grade water.
	Poor reagent delivery or evaporation during run	Check well-to-well volume variation. Repeat the assay for any sample with the wrong volume. Before each run, ensure the reaction plate is properly sealed. Refer to instrument guidelines to see if a compression pad is required.
	Air bubbles in reaction mix	Centrifuge reaction samples/plate prior to running on a real-time instrument.
	Poor mixing	Mix reactions thoroughly and re-run the PCR.
	Poor ROX signal	If you are using an instrument from Applied Biosystems then select ROX dye as the passive reference.
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 PCR instrument.