SensiMix™ Capillary Kit

Shipping: On Dry/Blue Ice

Batch No.: See vial

Concentration: See vial

Catalog Numbers

QT405-01: 100 x 20µl reactions: 1 x 400µl

QT405-05: 500 x 20µl reactions: 2 x 1ml

QT405-20: 2000 x 20µl reactions: 8 x 1ml

BIOLINE

A Meridian Life Science® Company

Store at –20°C

Storage and Stability:
The SensiMix™ Capillary Kit is shipped on Dry/Blue Ice. All kit components should be stored at -20°C upon receipt. Excessive freeze/thawing is not recommended. Since SYBR Green I is light-sensitive, it is important to avoid prolonged exposure to light. When stored under optimum conditions, the reagents are stable for a minimum of 12 months from date of purchase.

Quality Control:
Bioline operates under ISO 9001 Management System. The SensiMix Capillary Kit and it's components are extensively tested for activity, processivity, efficiency, heat activation, sensitivity, absence of nuclease contamination and absence of nucleic acid contamination.

Safety Precautions:
Harmful if swallowed. Irritating to eyes, respiratory system and skin. Please refer to the material safety data sheet for further information.

Description

The SensiMix™ Capillary Kit is a high-performance real-time PCR reagent designed for superior sensitivity and specificity. The kit has been formulated for use with capillary-based technology and is compatible with the Roche Lightcycler 1.0 and 2.0 platforms. The SensiMix Capillary Kit employs a hot-start polymerase, for high PCR specificity and sensitivity. Since SensiMix is inactivated and possesses no polymerase activity during reaction set-up, the kit prevents non-specific amplification including primer-dimer formation.

SensiMix™ Capillary has been developed to provide the high sensitivity required for demanding assays and is suitable for use with SYBR® Green I or probe chemistry.

Kit components

Reagent	100 x 20µl reactions	500 x 20µl reactions	2000 x 20µl reactions
SensiMix Capillary (5x)	1 x 400µl	2 x 1ml	8 x 1ml
Enzyme Mix	1 x 150µl	1 x 750µl	4 x 750µl
50x SYBR Green I	1 x 100µl	1 x 500µl	4 x 500µl
50mM $MgCl_2$	1 x 1ml	1 x 1ml	2 x 1ml

Kit compatibility

The SensiMix Capillary Kit has been optimized for use with all capillary based instruments.

In addition, the SensiMix Capillary Kit can be used for either probe or SYBR based technologies as a separate tube of SYBR Green I dye is supplied. The SensiMix Capillary Kit is optimized for use on the real-time instruments listed in the following compatibility table.

Manufacturer	Model
Roche	Lightcycler®1.0 Lightcycler®2.0

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 tube containing amplified PCR product should not be opened in the PCR set-up area.

Probe based PCR:
These guidelines refer to the use of TaqMan 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 PCR. We strongly recommend taking the following points 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 (Tm) of approximately 58-60°C. The Tm of the probe should be approximately 10°C higher than that of the primers
- optimal amplicon length should be 80-150bp and should not exceed 400bp
- a final primer concentration of 400nM is suitable for most probe reactions, however to determine the optimal concentration we recommend titrating in the range of 0.3-1.0 µM
- use equimolar primer concentrations
- a final probe concentration of 200nM is suitable for most applications. We recommend that the final probe concentration is lower than the primer concentration
- if possible, when amplifying from cDNA use intron-spanning primers to avoid amplification from genomic DNA
- for allelic discrimination, the single nucleotide polymorphism target base should be centrally positioned in the probe sequence

SYBR based PCR:
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 (Tm) of approximately 60°C
- optimal amplicon length should be 50-150bp
- a final primer concentration of 250nM is suitable for most PCR conditions, however to determine the optimal concentration we recommend a primer titration in the range of 0.1–1µM
- 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 1μg of complex (e.g. eukaryotic) genomic DNA in a single PCR. We recommend using the Bioline ISOLATE Genomic DNA Mini Kit (BIO-53021) 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 100ng cDNA per reaction, however it may be necessary to vary this amount. To perform a two-step RT-PCR, we recommend using the Tetro cDNA Synthesis Kit (BIO-65042). For high yield and purity of RNA, use the Bioline ISOLATE RNA Mini Kit

$MgCl_2$: The $MgCl_2$ concentration in the 1x reaction mix is 3mM, which is optimal for DNA Polymerase in the majority of real-time PCR conditions. If necessary, titrate the $MgCl_2$ to a maximum of 5mM.

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), 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 20μl final reaction mix and can be scaled accordingly.

Suggested thermal cycling conditions: The following PCR conditions are suitable for SensiMix Capillary Kit with a majority of amplicons. However, the cycling conditions can be varied to suit different reactions or 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 acquire at the appropriate wavelength(s).

N.B. This product is not suitable for non-capillary based systems

Probe protocol

Reagent	Volume	Final concentration
SensiMix™ Capillary Mix	4μl	1x
Enzyme Mix	1.5μl	1x
10μM Forward Primer	0.8μl	400nM
10μM Reverse Primer	0.8μl	400nM
10μM Probe	0.4μl	200nM
H_2O	up to 15μl	
Template	5μl	
	20μl Final volume	

- **Standard cycling**

Cycles	Temperature	Time	Notes
1	*95°C	*10min	Polymerase activation
40	95°C	5sec	
	60°C	20sec	Acquire at end of step

SYBR Green I protocol

Reagent	Volume	Final concentration
SensiMix™ Capillary Mix	4μl	1x
Enzyme Mix	1.5μl	1x
50x SYBR® Green I solution	0.4μl	1x
10μM Forward Primer	0.4μl	200nM
10μM Reverse Primer	0.4μl	200nM
H_2O	up to 15μl	
Template	5μl	
	20μl Final volume	

- **Standard cycling**

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

***Non-variable parameter**

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 primers/probe design	Use primers/probe design software or validated assays. Test assay on a control template
	Incorrect concentration of primers/ probe	SYBR reaction: use primer concentration between 100nM and 1μM. Probe reaction: use primer concentration between 300nM and 1μM and probe concentration at least 2-fold lower
	Template degraded	Re-isolate your template from the sample material or use freshly prepared template dilution
	Primers/probe degraded	Use newly synthesized primers/probe
	Template contaminated with PCR inhibitors	Further dilute template before PCR or purify template and resuspend it in PCR grade H_2O
	Template concentration too low	Increase concentration used
	Cycling conditions not optimal	Increase extension/annealing times, increase cycle number, reduce annealing temperature

Troubleshooting Guide (Continued)

Problem	Possible Cause	Recommendation
No amplification trace AND No product on agarose gel	Error in instrument setup	Check that the acquisition settings are correct during cycling
Non-specific amplification product AND Primer-dimers	Suboptimal primers/probe design	Redesign primers/probe using appropriate software or use validated assays
	Primers/probe concentration too high	Test dilution series of primer concentrations until primer dimer/non-specific amplification products disappear
	Primers/probe concentration too low	For SYBR based reactions, titrate primers in the concentration range of 100nM - 1μM. For Probe based reactions, increase concentration of primer in 100nM increments, adjust probe concentration to half the primer concentration
	Primers/probe 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
	Extension time too long	Reduce extension time to determine whether non-specific products are reduced
Late amplification trace	Activation time too short	Ensure that the reaction is activated for 10min 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 design of primers/probe	Redesign primers/probe using appropriate software or use validated primers
	Primers/probe concentration too low	Increase concentration of primers/probe in 100nM increments
PCR efficiency below 90%	Extension time is too short	Increase extension time
	Primers/probe concentration too low	Increase concentration of primers/probe in 100nM increments
	Suboptimal design of primers/probe	Re-design primers using appropriate software or use validated primers
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_2O
	Non specific amplification with SYBR reaction 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

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	100ml 200ml	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	100g 500g	BIO-41026 BIO-41025
PCR Water	Ultra-pure (18.2MΩ) molecular biology grade water	10 x 10ml	BIO-37080
DEPC-treated Water	Deionized, high-quality molecular grade water treated with DEPC. Ideal for use in all RNA work	10 x 10ml 1 Liter	BIO-38030 BIO-38031

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