

# SensiMix™ Capillary One-Step Kit

Shipping: On Dry/Blue Ice	Catalog Numbers
Exp. Date: See vial	QT255-01: 25 x 20µl reactions: 1 x 1.250µl
Batch No.: See vial	QT255-02: 250 x 20µl reactions: 2 x 1.25ml
Concentration: see vial	QT255-05: 500 x 20µl reactions: 4 x 1.25ml
	QT255-20: 2000 x 20µl reactions: 16 x 1.25ml



A Meridian Life Science® Company

Store at -20°C

## Storage and Stability:

The SensiMix™ Capillary One-Step 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 One-Step 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:

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 One-Step Kit contains a ready-to-use reagent for capillary-based real-time RT-PCR for use on various real-time instruments. The SensiMix Capillary One-Step Kit provides high-performance, reproducible first strand cDNA synthesis and subsequent real-time PCR assays, using a single protocol. Both cDNA synthesis and PCR are performed in a single tube using gene-specific primers and either total RNA or mRNA. The kit combines a reverse transcriptase and a proprietary hot-start DNA polymerase, for high PCR specificity and sensitivity of the one-step RT-PCR. 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 Capillary One-Step Kit is provided as a 2x mastermix containing all the components necessary for reverse transcription and real-time PCR including dNTPs, stabilisers and enhancers. As a ready-to-use premix, only primers and template need to be added. In addition, SensiMix Capillary One-Step Kit includes the highly efficient RiboSafe RNase inhibitor, which prevents enzymatic degradation of the RNA.

## Kit components

Reagent	25 x 20µl reactions	250 x 20µl reactions	500 x 20µl reactions	2000 x 20µl reactions
SensiMix™ Capillary One-Step Kit (2x)	1 x 250µl	2 x 1.25ml	4 x 1.25ml	16 x 1.25ml
50x SYBR® Green I	1 x 10µl	1 x 100µl	1 x 200µl	4 x 200µl
50mM MgCl <sub>2</sub>	1 x 1ml	1 x 1ml	1 x 1ml	4 x 1ml
RNase Inhibitor (10U/µl)	1 x 20µl	1 x 200µl	1 x 400µl	4 x 400µl
DEPC-H <sub>2</sub> O	1 x 1.8ml	1 x 1.8ml	1 x 1.8ml	4 x 1.8ml

## Kit compatibility

The 5x SensiMix Capillary One-Step Kit has been optimized for use with all capillary based instruments (see below).

Manufacturer	Model
Roche	Lightcycler®1.0 Lightcycler®2.0

## General considerations

When handling RNA it is important to use RNase-free plasticware and reagents. We also recommend performing RNA work in an isolated area. To help prevent any carry-over DNA contamination, we recommend that separate areas are maintained for PCR 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.

## 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 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<sub>m</sub>) of approximately 58-60°C. The T<sub>m</sub> 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 (T<sub>m</sub>) 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 RNA template is intact and devoid of contaminating inhibitors for both reverse transcription and subsequent PCR. For high purity RNA, we recommend using the Bioline ISOLATE RNA Mini Kit (BIO-52043). Prior to use in RT-PCR, RNA suspensions and dilutions should be made in DEPC-treated Water (BIO-38030) to avoid any RNase-mediated degradation.

The recommended amount of template for one-step RT-PCR is dependent upon the type of RNA used. The following should be considered when using total RNA and isolated mRNA:

- **total RNA:** purified total RNA can be used in the range from 1pg to 1µg per 50µl reaction
- **mRNA:** purified mRNA can be used from 0.5pg per 50µl reaction

**MgCl<sub>2</sub>:** The MgCl<sub>2</sub> concentration in the 1x reaction mix is 3mM, which is optimal for both Reverse Transcriptase and SensiTaQ in the majority of real-time RT-PCR conditions. If necessary, we recommend titrating MgCl<sub>2</sub> to a maximum of 5mM.

**RT-PCR Controls:** It is important to detect the presence of contaminating genomic DNA that may affect the reliability of the data. Always include a no-template control, replacing the template with PCR-grade water.

## Procedure

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

**Suggested RT-PCR conditions:** The cycling conditions can be varied to suit customer or machine-specific protocols. The critical step of the RT-PCR is the 10-minute polymerase activation at 95°C. The detection channel on the real-time instrument should be set to acquire at the appropriate wavelength(s).

## Probe protocol

Reagent	Volume	Final concentration
SensiMix™ Capillary One-Step Mix	10µl	1x
10µM Forward Primer	0.8µl	400nM
10µM Reverse Primer	0.8µl	400nM
10u/µl RiboSafe RNase Inhibitor	0.4µl	0.2U/µl
10µM Probe	0.4µl	200nM
DEPC-H <sub>2</sub> O	up to 15µl	
Template	5µl	
<b>20µl Final volume</b>		

### • Standard cycling

Cycles	Temperature	Time	Notes
1	42°C	10min	Reverse transcription
1	*95°C	*10min	Polymerase activation
40	95°C 60°C	10s 30-60s	Acquire at end of step

## SYBR Green I protocol

Reagent	Volume	Final concentration
SensiMix™ Capillary Mix	10µl	1x
10µM Forward Primer	0.4µl	200nM
10µM Reverse Primer	0.4µl	200nM
50x SYBR® Green I solution	0.4µl	1x
10u/µl RiboSafe RNase Inhibitor	0.4µl	0.2U/µl
DEPC-H <sub>2</sub> O	up to 15µl	
Template	5µl	
<b>20µl Final volume</b>		

### • Standard cycling

Cycles	Temperature	Time	Notes
1	42°C	10min	Reverse transcription
1	*95°C	*10min	Polymerase activation
40	95°C 55-60°C 72°C	15s 15s 15s	Temperature depends on the T <sub>m</sub> 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 (see licensing information, clause 4).

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

## 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 <sub>2</sub> O
	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 <sub>T</sub> ) 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 <sub>2</sub> O
	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](mailto:tech@bioline.com)  
 UK: +44 (0) 20 8830 5300  
 Germany: +49 (0) 3371 681 229  
 USA: +1 508 880 8990  
 Australia: +61 (0)2 9209 4180  
 All Others Nations: Local distributor or email [tech@bioline.com](mailto:tech@bioline.com)

## Associated Products

Product	Description	Pack Size	Cat No.
ISOLATE Genomic DNA Mini kit	Rapid isolation of DNA from a variety of samples	10 Preps 50 Preps 250 Preps	BIO-52031 BIO-52032 BIO-52033
ISOLATE Plant DNA Mini kit	Rapid isolation of DNA from a variety of plant samples	10 Preps 50 Preps 250 Preps	BIO-52034 BIO-52035 BIO-52036
ISOLATE RNA Mini Kit	Fast and efficient isolation of extremely pure total RNA from a variety of samples	10 Preps 50 Preps 250 Preps	BIO-52039 BIO-52040 BIO-52041
ISOLATE Plant RNA Mini Kit	Fast and efficient isolation of extremely pure total RNA from a variety of plant samples	10 Preps 50 Preps 250 Preps	BIO-52042 BIO-52043 BIO-52044
TRIsure™	Quick isolation of high-quality RNA from a variety of sources for subsequent use in cDNA synthesis	100ml 200ml	BIO-38032 BIO-38033
Tetro cDNA Synthesis Kit	Fully optimized to generate maximum yields of full-length cDNA from RNA	30 Reactions 100 Reactions	BIO-65042 BIO-65043
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

### TRADEMARK AND LICENSING INFORMATION

- 1). Trademarks: SensiMix™ (Bioline Reagents Ltd), SYBR® (Molecular Probes), LightCycler™ (Roche).
- 2). Purchase of this product includes limited right to use the supplied amount of SYBR® Green I Stain patented by Molecular Probes, Inc.
- 3). Notice to Purchaser: Limited License. Use of this product may be covered by one or more of the following US patents: 6,127,155, 5,677,152 (claims 1 to 23 only), 5,773,258 (claims 1 and 6 only). The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.
- 4). These reagents are provided for use in PCR. No licenses to third party patents in respect of melt-profile analysis are provided. Furthermore, melt-profile analysis may require a third-party license.
- 5). SensiMix products are manufactured by Bioline Reagents Ltd.

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)33 7168 1229  
Fax: +49 (0)337168 1244

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