SuScript 1-Step SYBR qPCR Kit

Catalog No	Size
• RT01A045	50 Rxn
• RT01A046	200 Rxn

Description:

SuScript 1-Step SYBR qPCR Kit is a convenient one-step solution for the SYBR™ Green dye–based PCR amplification and detection containing all the components necessary to obtain cDNA in a high yield from RNA samples and SYBR-based qPCR amplification with hotstart activity. The RNase Inhibitor presented in the kit adequately protects RNA templates against degradation. Optimized reaction buffer, $MgCl_2$, Oligo-dTs, random hexamers and reverse transcriptase are also included in the 5X RTMix. The first strand of cDNA can be directly used as a template in downstream applications (PCR, Real-Time PCR, etc).

Kit Components:

Components	RT01A035	RT01A036
5X RTMix	200 µl	800 µl
2X SuYBRGreen qPCR Mastermix	1 ml	4 ml

General Considerations:

- High purity template RNA is essential for reliable efficient cDNA synthesis. A A260 / A280 ratio of 1.7 or higher is strongly recommended.
- The amount of template RNA is depended on the expected copy number of the sequence of interest. In general, 1 µg – 1 ng of total RNA is recommended, 0.05 – 100 ng if you are working with isolated mRNA.
- This protocol recommends cDNA synthesis for 1 hour at 42°C.
- To enhance the template coverage, the 5X RTMix also contains random hexamer primers. This provides multiple priming sites along the RNA for the detection of multiple short sequences.

Recommended Protocol:

This protocol is exemplary for one reaction, and for multiple reactions it is necessary to calculate the components in a proportional manner. Mix the kit components in a micro tube in the recommended proportions below. Briefly centrifuge the tube to spin down the contents and to eliminate any air bubbles. Place the tube on ice until to reverse transcriptase reaction.

Reaction Components	Volume
5X RT Mix	4 µl
Total RNA	1-6 µl
DNase/RNase Free Water	Up to 20 µl
Total Volume	20 µl

Mix gently and carry-out reverse transcriptase reaction conditions:

RT Steps	Temp (°C)	Time	Cycle
RT Reaction	42	1 hour	1
RT Deactivation	80	10 min	1

Notice: The synthesized cDNA can be used immediately, without purification, or stored at -20 °C for future use.

Reaction Setup:

- It is recommended that thaw the 2X SuYBRGreen qPCR Mastermix and mix throughly but gently to ensure even distribution of the components.
- Put the specimen's DNA on the PCR tubes or multi-well plate.
- For negative control sample do not add DNA please use nuclease-free water.
- Keep plate or PCR tubes on ice during reaction set up.

Components	Volume
2X SuYBRGreen qPCR Mastermix	25 µl
Forward primer (10 µM)	1 µl
Reverse primer (10 µM)	1 µl
Sample DNA (max 10 µl)	2 µl
Nuclease-free water	X to complete 50 µl
TOTAL Volume	50 µl

qPCR Conditions:

Steps	Temp.	Time	Cycles
HotStart Activation	95 ℃	5 min	1
Amplification	95 ℃	10 sec	40
	55-60 ℃	15 sec	
	72 ℃	20 sec (reading)	

Storage Conditions:

Store all contents at -20 ºC in a freezer.

Quality Control:

Nicking activity, priming activity, exonuclease activity, or endonuclease activity has not been detected.

Evaluation of Results:

Analyze the amplification of the samples in gel electrophoresis, positive and negative controls for any band observed. If the amplicon band is observed in Positive Control but doesn't in Negative Control, amplification reaction was completed properly. If any other situation occurs, however, amplification reaction may be proceeding in a wrong way. In such a case, re-test the samples from reagent preparation or check the primer design.

SuGenomik Biyoteknoloji Ltd Şti

Address: METU Technopolis, Ankara.

Web: www.sugenomik.com

Mail: info@sugenomik.com

Tel: +90(312)385 85 89