FastSYBR Mixture

Cat. #: W0955-10 (10 ml) W0955-25 (25 ml)

Store at -20°C, if used frequently, store at 2-8°C to avoid repeated freezing and thawing. Ship at 4°C

Product Introduction:
The FastSYBR Mixture is a premixed system for real-time fluorescence quantitative PCR (SYBR Green I), and the concentration is 2×. It contains Fast Taq DNA Polymerase, PCR Buffer, dNTPs, SYBR Green I Fluorescent Dye, and Mg^{2+}. The operation is simple and convenient. This product is mainly used for the detection of genomic DNA target sequences and cDNA target sequences after RNA reverse transcription.

This product contains the fluorescent dye SYBR Green I which can bind with all double-strand DNA, so that the product can be used for the detection of different target sequences without the need for the synthesis of specific labeled probes. The Fast Taq DNA Polymerase in the mixture is a chemically-modified, new efficient enzyme which reduce non-specific amplification efficiently, and it is activated by incubation at 95°C for 20 seconds, which will efficiently reduce the total reaction time.

This product is suitable for fluorescent qPCR instruments with no ROX. This product is for research use only.

No ROX as a calibration dye: Roche LightCycler 480, Roche LightCyler 96, Bio-rad iCyler iQ, iQ5, and CFX96.

Need Low ROX (30-50mM) as a calibration dye: ABI Prism7500/7500 Fast, QuantStudio® 3 System, QuantStudio® 5 System, QuantStudio® 6 Flex System, QuantStudio® 7 Flex System, ViiA 7 system, Stratagene Mx3000/Mx3005P, Corbett Rotor Gene 3000

Need High ROX (300-500mM) as a calibration dye: ABI Prism7000/7300/7700/7900， Eppendorf， ABI Step One/Step One Plus.

Features: This product uses a new high-performance hot start enzyme (GoldStar Taq DNA Polymerase) and a unique PCR buffer system. This product significantly improves the PCR amplification efficiency and has high sensitivity and specificity. This product is suitable for quantitative PCR detection and can accurately quantify and detect the target gene.

Precautions:

1. Mix gently before use, avoid foaming, and use after brief centrifugation.
2. This product contains SYBR Green I fluorescent dye. Avoid strong light irradiation when storing this product or preparing PCR reaction solution.
3. Avoid repeated freezing and thawing of this product. Repeated freezing and thawing may comprise product performance.
4. This product cannot be used for qPCR using probes.
5. When preparing the reaction solution, use new or non-contaminated tips and centrifuge tubes to prevent contamination.

Protocol:

The following protocol is an example of conventional PCR reaction system and condition. The actual protocol should be improved and optimized based on the template, primer structure and the size of the target.

1. PCR reaction system:

Reagent	50 µl	Final Conc.
2× FastSYBR Mixture	25 µl	1x
Forward Primer, 10 µM	1 µl	0.2 µM
Reverse Primer, 10 µM	1 µl	0.2 µM
DNA template	2 µl	
ddH_2O	Up to 50 µl	

Note:

1) Usually 0.2 µM of primer concentration gives better results, and the final concentration of primers should be between 0.1 and1.0 µM.

2) Usually the amount of DNA template is 10-100 ng for genomic DNA or 1-10 ng for cDNA. Template can be gradient diluted to optimize.

3) The recommended reaction volume is 50 µl, and the reaction volume can also be scaled up or down according to actual experimental requirements.

2. PCR reaction program:

   It is recommended to use two-step PCR reaction program. If a good result cannot be obtained due to the low Tm of the primers, try a three-step PCR program.

Procedure	Temperature	Time	
Pre-denaturation	95°C	20 sec	
Denaturation	95°C	3 sec	} 35-40 cycles
Annealing/Extension	60°C	30 sec	
Melting curve analysis			
	95°C	15 sec	
	60°C	1 min	
	95°C	15 sec	
	60°C	15 sec	

Note: 1) The annealing temperature should be between 60-64°C. If there is non-specific reaction, increase the annealing temperature.

2) The melting curve analysis should be set as the procedure recommended by qPCR instrument used.

Optimization of reaction conditions: When optimizing the qPCR reaction conditions, different aspects such as the concentration of the primer, the annealing temperature, and the extension time should be considered, to improve the reaction specificity and amplification efficiency.

1. The experimental system with high reaction specificity and high amplification efficiency should be as the following conditions:
   1) High specificity: no non-specific amplification such as primer dimers for negative control; No other amplification beyond the target fragment.
   2) High amplification efficiency: Low Ct value; Amplification efficiency of PCR is high, close to the theoretical value of 100%.
2. Methods of optimization of reaction conditions:
   1) Primer concentration: Usually 0.2 μM of primer concentration gives better results, and the final concentration of primers should be between 0.1 and1.0 μM. To increase the specificity of the reaction, decrease the concentration of the primer; To increase the amplification efficiency, increase the concentration of the primer.
   2) Annealing temperature: It is recommended to use two-step PCR, and set the annealing temperature as 60°C. To increase the specificity, increase the annealing temperature, which should be between 60-64°C. If a good result cannot be obtained due to the low Tm of the primers, try a three-step PCR program. The annealing temperature of the three-step PCR program should be between 56°C and 64°C.
   3) Extension time: It is recommended to use two-step PCR and set the extension time as 1 minute. To increase amplification efficiency, increase the extension time, or try three-step PCR.

   Three-step QPCR method

Procedure	Temperature	Time	
Pre-denaturation	95°C	20 s	
Denaturation	95°C	3 s	35-40 cycles
Annealing	56-64°C	30 s	
Extension	72°C	32 s	
Melting curve analysis			
	95°C	15 s	
	60°C	1 min	
	95°C	15 s	
	60°C	15 s	

Note:

(1) If good amplification efficiency cannot be achieved, lower the annealing temperature appropriately. If there is non-specific reaction, increase the annealing temperature.

(2) To increase amplification efficiency, increase extension time appropriately.

(3) The melting curve analysis should be set as the procedure recommended by qPCR instrument used.

Our customers also buy:

Better than Matrigel	*Real 3D* cell culture gel, 3-stiffness & save Cat.# P720
Mouse Tail DNA Extract	20 min. done. Accurate & consistent. No use of organic solvents. Ready for PCR Cat.# T605
1 drop, do PCR Mix	PCR Master Mix - easy dripping Cat.# W2599-5
Thermophilic Reverse Transcriptase	with optimal temperatures at 60-62°C, it can be heat-inactivated at ⩾90°C. Cat.# W140
	1-Step 2x RT-PCR Master Mix-SYBR # W141, W142, W143 *Make RNA analysis easy as DNA analysis.*
10X Virus Titer-up Boost Cat.# P906 / P909	Regulates packaging in transcriptional level Increases Lenti/Retro viral particles up to 10-fold
Water Bath Treatment	1 tablet / treatment / week Cat.# T20
Simple, Reliable	SV40T / hTERT *Cell Immortalization Kits*
2× Flash HS PCR MasterMix (Dye)	fast extension rate (5-15 sec/kb) and fast HotStart Cat.# W3007-1