Product Information

SYBR Green I 10,000× in DMSO

Catalog Number: S2018

Product Size: 0.5 mL

Storage

Store at 4°C and protect from light. When stored as directed, product is stable for at least 12 months.

Description

SYBR Green I is a commonly used fluorescent dye that binds double-stranded DNA molecules by intercalating between the DNA bases. It is used in quantitative PCR because the fluorescence can be measured at the end of each amplification cycle to determine, relatively or absolutely, how much DNA has been amplified.

SYBR Green I is one such fluorescent DNA-binding dye that can be included in PCR buffer. When bound to DNA, the dye chances structurally and becomes less mobile, causing its energy to be released as fluorescence. The fluorescence increases along with the concentration of DNA.

SYBR Green I has an excitation and emission maxima of 494 nm and 521 nm, respectively.

Protocol

1. Before use, thaw at room temperature and mix well by gentle vortexing. After thawing, the master mix should be kept on ice before use. It can be refrozen for storage.

2. Use DMSO or dH_2O to dilute 10,000 × dye 500 times for subsequent experiments.

3. Set up the PCR reaction (For reference only)

Name	volume
10× polymerase buffer	5 µL
without magnesium	
50 mM $MgCl_2$	2.5 µL
2 mM dNTP	5 µL
20× SYBR Green I	2.5 µL
Taq DNA polymerase	1-5 units
F, R Primers	0.1-0.5 µM each of primers (final concentrations)
DNA	appropriate amount
dH_2O	to a final volume of 50 µL

Note: The amount of DNA added is usually less than 100 ng. Because different kinds of DNA contain different copies of target genes, gradient dilution can be carried out if necessary to determine the appropriate amount of DNA template addition. The amount of cDNA added as template should not exceed 10% of the total volume of the PCR reaction solution.

4. Perform real-time quantitative PCR program to collect fluorescent signals.

Cycling Step	Temperature	Holding Time	Number of Cycles
Enzyme Activation	95 ℃	2 min	1
Denaturation	95 ℃	5 s	45
Annealing	50-60 ℃	5 s	
Extension	72 ℃	25 s	

5. Analyze experimental data.

Notes

1. The concentration of SYBR Green I is a key factor of real-time PCR experiments. Too low will reduce the change in fluorescence signal, resulting in low-copy samples that may not

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be detectable, and at high concentrations it will inhibit the PCR reaction. Therefore, when using SYBR Green I dye, the concentration should be optimized according to the actual situation. The final reaction concentration is between 1× and 0.2×.

2. Increasing the concentration of Mg^{2+} can reduce the inhibitory effect of SYBR Green I on the PCR reaction. We recommend that when using SYBR Green I for real-time PCR experiments, the concentration of Mg^{2+} is 0.5 to 3 mM higher than that of ordinary PCR without SYBR Green I.

3. For your safety and health, please wear lab coats and disposable gloves.

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