SYBR green I chloride

Cat. No.: HY-D1191

CAS No.: 2225748-05-8

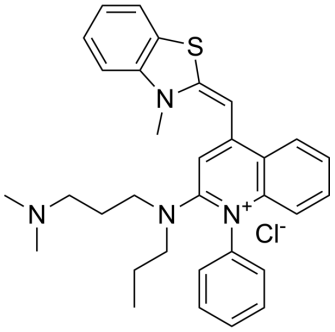
Molecular Formula: $C_{32}H_{37}ClN_4S$

Molecular Weight: 545.18

Target: Fluorescent Dye; DNA/RNA Synthesis

Pathway: Others; Cell Cycle/DNA Damage

Storage: 4°C, sealed storage, away from moisture and light
* In solvent : -80°C, 6 months; -20°C, 1 month (sealed storage, away from moisture and light)



SOLVENT & SOLUBILITY

In Vitro

DMSO : 50 mg/mL (91.71 mM; Need ultrasonic)

Preparing Stock Solutions	Mass / Solvent / Concentration	1 mg	5 mg	10 mg
	1 mM	1.8343 mL	9.1713 mL	18.3426 mL
	5 mM	0.3669 mL	1.8343 mL	3.6685 mL
	10 mM	0.1834 mL	0.9171 mL	1.8343 mL

Please refer to the solubility information to select the appropriate solvent.

BIOLOGICAL ACTIVITY

Description

SYBR Green I chloride is a highly sensitive fluorescent nucleic acid dye that binds specifically to the minor groove of double-stranded DNA or intercalates between base pairs. SYBR Green I chloride exhibits weak fluorescence in the unbound state but emits bright fluorescence upon binding, and it preferentially binds to large-fragment DNA and DNA with high G+C content. SYBR Green I chloride is suitable for real-time PCR technology; its fluorescence intensity correlates with the amount and size of amplification products, enabling accurate quantification of gene expression and discrimination of amplicons via melting curve analysis without additional post-processing. SYBR Green I chloride is widely used in preclinical in vitro nucleic acid detection[1][2].

In Vitro

Real-Time RT-PCR for Cytokines[1]
1. Sample and Nucleic Acid Preparation
Cell treatment:
Splenic monocytes are isolated from mice and divided into a control group and a PMA-ionomycin stimulation group, followed by incubation at 37℃ with 5% CO_2 for 5 h.
RNA extraction:
Cells are lysed with TRIZOL to extract total RNA; genomic DNA is removed with DNase I, RNA integrity is verified via denaturing gel electrophoresis, and RNA is quantified at 260 nm.

cDNA synthesis:
2 μg of RNA is annealed with 1 μg of oligo-dT; a reverse transcription system is added, and cDNA is synthesized through the procedure of 25℃ for 5 min → 37℃ for 60 min → 70℃ for 10 min.
2. Reaction System Preparation (50 μL total volume)
The system contains 0.5×SYBR Green I (10000× stock solution), PCR buffer (50 mM KCl, 20 mM Tris-HCl pH 8.3, 2.5 mM $MgCl_2$, 0.2% glycerol, 0.2% DMSO), 0.2 mM forward and reverse primers, 0.2 mM dNTPs, 1.25 U AmpliTaq, and 5 μL of 1:5 diluted cDNA.
No-template controls (NTC) are set up, and all samples are run in at least 2 replicates.
3. Amplification Cycling Program
Pre-denaturation is performed at 95℃ for 30 s; 35 cycles of amplification are carried out (95℃ for 30 s → 60℃ for 30 s → 72℃ for 1 min → 85℃ for 5 s).
Fluorescence collection:
Fluorescence is collected at 85℃ during each cycle (a temperature higher than the Tm of primer dimers and lower than the Tm of specific amplicons).
4. Melting Curve Analysis
After amplification, the temperature is increased from 60℃ to 95℃ at a rate of 2℃/min, and fluorescence is detected every 15 s.
Target amplicons and non-specific signals from primer dimers are distinguished based on specific Tm peaks.
5. Quantification and Specificity Verification
Data analysis:
The baseline is set to cycles 1-5, with a threshold of 0.7; Ct values are read by software, absolute copy numbers are calculated using an external plasmid standard curve (10^9-10^4 copies); β-actin is used as an internal reference for normalization to calculate relative expression levels.
Specificity verification:
A single target band is confirmed via 2% agarose gel electrophoresis, which matches the specific peak in the melting curve.
Multiplex Real-Time PCR Based on Bacterial Samples[2]
1. Template Preparation
Strain culture: *Vibrio cholerae* is inoculated onto Columbia blood agar and cultured aerobically at 35°C for 24 h; *Legionella pneumophila* is inoculated onto BCYE agar and cultured aerobically at 35°C for 3 days.
DNA extraction: For *Vibrio cholerae*, 2-3 colonies are resuspended in 200 μL Instagene, and DNA is prepared following the kit instructions; for *Legionella pneumophila*, a bacterial suspension with a turbidity of 0.5 McFarland standard is prepared as the template.
2. Reaction System Preparation (20 μL total volume)
Homemade SYBR Green I (SG) premix:
1× PCR Buffer II, 4 mM $MgCl_2$, 200 μM dNTP, primers (0.5 μM each of *Legionella* 16S rRNA primers, 0.1 μM each of mip primers; 0.5 μM each of singleplex mip primers), 1 U AmpliTaq Gold, SG at a final concentration of 0.2× or 0.4× (from 10000× stock solution), and 4 μL template DNA.
3. Amplification Cycling Program
For *Vibrio cholerae*: Pre-denaturation at 95°C for 10 min; 40 cycles of 94°C for 20 s → 60°C for 45 s → 72°C for 45 s.
For *Legionella pneumophila*: Pre-denaturation at 95°C for 10 min; 30 cycles of 94°C for 10 s → 50°C for 20 s → 72°C for 25 s.
Fluorescence collection: Performed at 72°C in the SYBR channel (excitation at 470 nm, detection at 585 nm) with gains set to 2 and 5.
4. Melting Curve Analysis
After amplification, the temperature is increased from 75°C to 99°C at a rate of 1°C per 60 s.
Software analysis: RotorGene V4.6.94 is used with digital filtration set to none, and the Tm value of amplicons is read.
5. Product Verification
1% agarose gel electrophoresis (with Gelstar dye added) is performed, and the melting curve is compared with the electrophoresis bands to verify the amplification of multiplex products.
6. Key Usage Points
In multiplex PCR, an SG concentration of **≥0.4×** is required for simultaneous detection of multiple amplicons; a concentration of 0.2× only detects products with high GC% and large fragment sizes.

CUSTOMER VALIDATION

- J Pharm Anal. 2025 Dec 11.

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REFERENCES

[1]. Ramos-Payán R, et al. Quantification of cytokine gene expression using an economical real-time polymerase chain reaction method based on SYBR Green I. Scand J Immunol. 2003;57(5):439-445.

[2]. Giglio S, et al. Demonstration of preferential binding of SYBR Green I to specific DNA fragments in real-time multiplex PCR. Nucleic Acids Res. 2003;31(22):e136.

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