SYBR® Green staining reagent, DNA free

10x concentrated SYBR® Green I staining solution, DNA-free
Product code A8511

Description

SYBR® Green is an asymmetrical cyanine dye. It is used as an intercalating dye for the general detection of double-stranded DNA (dsDNA). Our 10-fold concentrated DNA-free SYBR® Green I dye solution is particularly suitable for qPCR using general primers such as 16S rDNA or 18S rDNA primers.
An additional application is the staining of DNA in gel electrophoresis. SYBR® Green shows lower mutagenic potential in comparison to ethidium bromide (1). Thus, SYBR® Green is often used as a substitute to the classical Ethidium bromide dye. Nevertheless, follow the usual safety precautions dealing with DNA dyes.
Synergistic effects have been shown to increase the mutagenicity of the dye (2).
The complex of DNA and SYBR® Green absorbs blue light of wavelength 494 nm (absorption maximum) and emits green light at 521 nm (emission maximum). The stained DNA can be detected on a blue light transilluminator. Other absorption maxima in the UV range are at 284 nm and 382 nm. Hence, SYBR® Green stained DNA can also be detected on the UV transilluminator.

Pack size:

Product code A8511,50625 5 vials of 0.625 ml

Literature

(1) Singer VL, Lawlor TE, Yue S. (1999) Comparison of SYBR Green I nucleic acid gel stain mutagenicity and ethidium bromide mutagenicity in the Salmonella/mammalian microsome reverse mutation assay (Ames test). *Mutation Research 439: 37-47.*
(2) Ohta T, Tokishita S, Yamagata H (2001) Ethidium bromide and SYBR Green I enhance the genotoxicity of UV-irradiation and chemical mutagens in *E. coli. Mutation Research 492: 91–97.*

Storage:	-20°C Protected from light. Avoid repeated freeze-thaw cycles! We recommend storing the product at 2-8°C after its first use.
Specification:	Bacterial DNA: not detectable (min. 40 PCR cycle) Concentration: 10X concentrated solution

Protocols

A) Real-Time PCR with SYBR® Green staining reagent, DNA-free

Materials required: Real-Time/qPCR instrument, PCR tubes, and strips. Devices: for example StepOne™, CFX, or LightCycler®.

Note: Disposable plastics (PCR tubes, pipette tips, polypropylene tubes) that get in contact with the PCR must be DNA-free. Make sure to run all steps in DNA-free environment (use DNA/RNA-ExitusPlus™-treated and /or UV irradiated).

- Thaw staining solution slowly (on ice). Keep staining solution in the dark until use.
- Set all reagents on ice (mastermix, DNA-free water, DNA-free *Taq* DNA polymerase, etc.)
- Mix the tubes on a vortex mixer and centrifuge briefly.

For PCR volumes of 25 µl:

1. Set x µl DNA-free water in each vessel prior to PCR (corresponding to a final volume of 25 µl). Keep vials chilled.
2. Add PCR buffer ($MgCl_2$ solution), primer, and *Taq* DNA polymerase, individually or as a mastermix*.
3. Add 2.5 µl of SYBR® Green staining reagent, DNA-free.
4. Add template DNA. (Keep vials chilled until transferred to the thermocycler block.)
5. Start the PCR program.

*for PCR series the preparation of a mastermix is recommended.
For example: for 10 reactions (25 µl volume and the final addition of 2 µl DNA template) pipette in a DNA-free vessel the following reagents:

95 µl DNA-free water
100 µl of a 2.5X PCR buffer (with $MgCl_2$)
5 µl forward primer (10 µM)
5 µl reverse primer (10 µM)
25 µl SYBR® Green staining reagent, DNA-free
8 µl *Taq* DNA polymerase DNA-free
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238 µl total volume of mastermix

- Mix by pipetting up and down or vortex for 5 sec. Spin very briefly in a centrifuge.
- Pipette 23 microliters of this mastermix in each PCR tube (keep refrigerated all time!)
- Add 2 µl of template DNA or 2 µl of DNA-free water (for negative controls), respectively.

With each Real Time PCR-series run a positive control (e.g. extracted from bacteria culture) containing 10 to 100 ng of the standard DNA.

Real-Time PCR thermocycler conditions:
Set internal reference ROX off before the PCR run.
For Real-Time PCR instruments from Applied Biosystems: select 1 as the appropriate channel for SYBR® Green detections.

For T_m analysis:
Read 70 - 95°C in 0.2°C increments; hold 1 sec between measurement points.

B) Staining Gels after Electrophoresis using SYBR® Green staining reagent, DNA-free

SYBR® Green can be used for staining of dsDNA on agarose and polyacrylamide gels. (In polyacrylamide gels signals are more intense compared to ethidium bromide-stained gels).

- Thaw the staining solution slowly (on ice). Keep the staining solution in the dark until use.

1. For gel analysis, mix 9 µl of the PCR solution (PCR product) with 1 µl of SYBR® Green staining reagent in a reaction vessel or in the well of a 96-well plate.
2. Incubate for 15 min in the dark (!) to allow for the binding of the dye to DNA fragments.
3. Add 2 µl of a 6X gel-loading buffer. Put the finished mixture (total volume 12 µl) into the slots of a gel (apply DNA size marker accordingly).
4. The subsequent gel documentation is carried out on an UV table, as for Ethidium bromide. Alternatively, SYBR® Green-stained DNA may be visualized on a blue light transilluminator.

Related products:

Solutions for the removal of nucleic acids contamination in the PCR laboratory:

DNA/RNA-ExitusPlus™	Product code A7089
DNA/RNA-ExitusPlus™ IF	Product code A7409