SYBR® Safe DNA Gel Stain

Table 1. Contents and Storage Information.

Material	Amount	Concentration	Storage	Stability
SYBR® Safe in TBE buffer	1 L * or 4 L †	0.5X, 45 mM Tris-borate, 1 mM EDTA, pH ~8.3	• Room temperature • Protect from light	~6 months when stored as directed
SYBR® Safe in TAE buffer	1 L * or 4 L †	1X, 40 mM Tris-acetate, 1 mM EDTA, pH ~8.3		
SYBR® Safe in DMSO	400 µL	10,000X		
SYBR® Safe DNA Gel Stain Starter Kit	1 L of SYBR® Safe gel stain, and one SYBR® Safe photographic filter	0.5X TBE		

Number of Labelings: * Provides sufficient material to stain ~20 minigels. † Provides sufficient material to stain ~80 minigels. 4 L unit size is packaged in a cube-shaped container with a removable spigot for easy dispensing and storage.

Spectral Data: 280, 502/530 nm, bound to DNA

Introduction

SYBR® Safe DNA gel stain has been specifically developed for reduced mutagenicity, making it safer than ethidium bromide for staining DNA in agarose or acrylamide gels. SYBR® Safe stain comes either as a concentrate or as a ready-to-use solution that can be used just like an ethidium bromide solution, and the detection sensitivity with SYBR® Safe stain is comparable to that obtained with ethidium bromide. DNA bands stained with SYBR® Safe DNA gel stain can be detected using a standard UV transilluminator, a visible-light transilluminator, or a laser-based scanner. The stain is also suitable for staining RNA in gels. Bound to nucleic acids, SYBR® Safe stain has fluorescence excitation maxima at 280 and 502 nm, and an emission maximum at 530 nm (Figure 1).

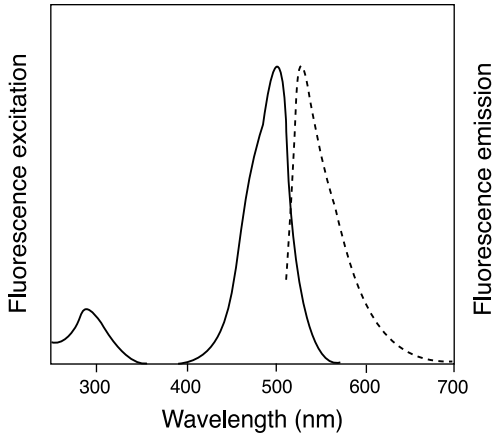

Figure 1. Normalized fluorescence excitation and emission spectra of SYBR® Safe DNA gel stain, determined in the presence of DNA.

Before You Begin

Handling and Disposal

SYBR® Safe DNA gel stain showed no or very low mutagenic activity when tested by an independent, licensed testing laboratory, and this stain is not classified as hazardous waste under U.S. Federal regulations. The safety testing included three well-established mammalian cell–based tests (Table 2), a battery of well-established Ames-test bacterial strains (Figure 2), and extensive testing for environmental safety (Tables 3 and 4). Nevertheless, please exercise appropriate care and judgment when using this reagent, and dispose of the stain in compliance with all pertaining local regulations.

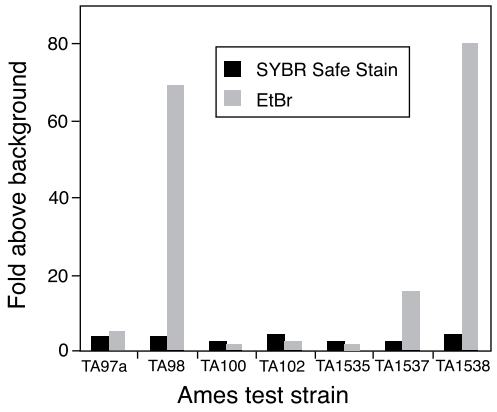

Figure 2. Summary of Ames test results for mutagenicity. Samples were pre-treated with a mammalian S9 fraction and then tested using the indicated Ames test strain. With strains TA97a, TA98, TA100, and TA102, a result of less than twofold above background suggests that the compound is nonmutagenic in the test; whereas, a result of greater than this value suggests that the compound is mutagenic in the test. With strains TA1535, TA1537, and TA1538, a result of less than threefold above background suggests that the compound is nonmutagenic in the test; whereas, a result of greater than this value suggests that the compound is mutagenic in the test. All tests were performed by Covance Laboratories, Inc., Vienna, VA, an independent testing laboratory.

Table 2. Summary of mammalian cell–based tests for DNA genotoxicity.

Test *	Cell Type	Test Result with S9 Activation †	Test Result without S9 Activation †
Transformation test [1]	Syrian hamster embryo (SHE) cells	Not applicable	Negative
Chromosomal aberration test [2]	Cultured human peripheral blood lymphocytes	Negative	Negative
Forward-mutation test [3,4]	L5178Y TK mouse lymphoma cells	Negative	Negative

***** All tests were performed by Covance Laboratories, Inc., Vienna, VA, an independent testing laboratory. **†** S9, a mammalian extract obtained from Aroclor™ 1254-induced rat liver.

1. Fundamental and Molecular Mechanisms of Mutagenesis 356:1 (1996); **2.** Evans, H.J., in *Chemical Mutagens, Principles and Methods for their Detection Vol 4,* A. Hollaender, Ed., Kluwer Academic/Plenum Publishers (1976) pp. 1–29; **3.** Mutation Res 72, 447 (1980); **4.** Mutation Res 59, 61 (1979).

Table 3. Summary of environmental safety test results.

Analysis *	Method	Results
Aquatic toxicity	Fathead minnow CA Title 22 acute screening	Not classified as hazardous or toxic to aquatic life
Ignitability	EPA 1010	Not ignitable (>212ºF)
Corrosivity	EPA 150.1	Not corrosive (pH = 8.25)
Corrosivity (by Corrositex)	DOT-E 10904	Category 2 noncorrosive
Reactivity	EPA 9010B/9030A	No reactivity detected

***** All tests were independently confirmed by AMEC Earth and Environmental San Diego Bioassay Laboratory, San Diego, CA.

Protocols

Staining Nucleic Acids after Electrophoresis

1.1 Soak the gel in SYBR® Safe stain. If using SYBR® Safe gel stain concentrate, dilute 10,000X in TAE or TBE buffer (as appropriate) prior to use. Place the gel in a plastic container, such as a pipet-tip box lid or a household food-storage container. Do not use a glass container, as the dye in the staining solution may adsorb to the walls of the container, resulting in poor gel staining. Add sufficient SYBR® Safe DNA gel stain to cover the gel. A 50 mL volume is sufficient for staining most standard minigels. To stain larger gels, increase the volume of staining solution in proportion to the increased gel volume, and ensure that the entire gel is fully immersed during staining.

1.2 Incubate for 30 minutes. Protect the gel and staining solution from light by covering it with aluminum foil or by placing it in the dark. Gently and continuously agitate the gel at room temperature (e.g., on an orbital shaker at 50 rpm). No destaining is required.

Precasting SYBR® Safe Stain in Agarose Gels

2.1 Prepare the agarose gel directly in SYBR® Safe DNA gel stain. SYBR® Safe stain is provided in buffer; simply substitute SYBR® Safe stain for the buffer when preparing the molten agarose. If using the 10,000X SYBR® Safe stain concentrate, dilute the concentrated stain 1:10,000 in agarose gel buffer (e.g., 1X TBE or 1X TAE) and add the buffer plus stain mixture to the powdered agarose. For example if you run TBE gels and require 30 mL of molten agarose for your tray, add 3 µL of 10,000X SYBR® Safe stain concentrate to 30 mL of 1X TBE and mix well. Add this stain–TBE mixture to the powdered agarose.

The agarose/SYBR® Safe stain mixture may be heated in the microwave. As with precasting gels with ethidium bromide, the mobility of nucleic acid fragments in the gel may be somewhat slower when run in these gels, compared to their mobility in the gel without stain.

2.2 Run the gel. Use a running buffer appropriate to the SYBR® Safe gel stain formulation. No post-staining or destaining is needed.

Viewing and Photographing the Gel

Stained gels can be viewed using a standard 300 nm transilluminator, a 254 nm epi- or transilluminator, or a blue-light transilluminator such as the Safe Imager™ blue-light transilluminator from Molecular Probes (S37102). DNA stained with SYBR® Safe stain can also be visualized and analyzed using imaging systems equipped with an excitation source in the UV range or between 470–530 nm. Refer to Table 5 to determine the optimal filter sets to use, or contact the instrument manufacturer for advice.

Note: If bands from the SYBR® Safe stained gel are to be excised and used in a ligation reaction, we recommend that the gel is illuminated using blue-light source (i.e., Safe Imager™ blue-light transilluminator) and not a UV light source. In some instances, UV light sources in combination with SYBR® Safe stain can lead to reduced cloning efficiencies.

Stained gels can be photographed using Polaroid® 667 black-and-white print film and SYBR® Safe photographic filter (S37100). Molecular Probes' SYPRO® photographic filter (S6656) or a Kodak® Wratten #9 filter will also work well. Using this film and one of these filters, SYBR® Safe DNA gel stain provides the same detection sensitivity as ethidium bromide using a photographic filter appropriate for ethidium bromide. A standard ethidium bromide photographic filter may not be appropriate for use with SYBR® Safe DNA gel stain. Gels stained with SYBR® Safe stain can also be imaged using a CCD camera or a laser-based scanner.

Table 4. Summary of pollutant discharge test results.

Test (method, per CFR Title 40, Part 136) *	SYBR Safe Stain in 0.5X TBE †	0.5X TBE
pH (150.1)	8.45	8.48
Total cyanide (335.2)	None detected	None detected
Chemical oxygen demand (COD; 410.1)	7020	6840
Ammonia as nitrogen (350.1)	253	248
Total organic carbon (415.1)	2480	2360
Total phenolics (420.1)	None detected	None detected
Organochlorine pesticides and PCBs (608M)	None detected	None detected
Semi-volatile organic compounds (625)	None detected	None detected
Volatile organic compounds (624)	None detected	None detected
Metals (6010B, 7060A, 7421, 7470A, 7740, 7841)	None detected	None detected

* CFR = Code of Federal Regulations; † 1X SYBR® Safe stain (Lot X40023) in 0.5X TBE.

Table 5. Filter selection guide for use with SYBR® Safe stain.

Instrument (Manufacturer)	Excitation Source	Emission Filter
AlphaImager (Alpha Innotech)	302 nm	SYB-500
AlphaImager HP (Alpha Innotech)	302 nm	SYB-500
AlphaDigiDoc RT (Alpha Innotech)	UV transilluminator	
Shroud, Camera Stand (Alpha Innotech)	UV transilluminator	SYB-100
DE500 or DE400 light cabinet 2.17″ diam. (Alpha Innotech)	UV transilluminator	SYB-500
DE500 or DE400 light cabinet 2″ diam. (Alpha Innotech)	UV transilluminator	SYB-400
VersaDoc Imaging Systems (Bio-Rad)	Broadband UV	520LP
Molecular Imager FX Systems (Bio-Rad)	488 nm	530 nm BP
Gel Doc Systems (Bio-Rad)	302 nm	520DF30 (#170-8074)
Typhoon 9400/9410 (GE Healthcare)	488 nm	520 BP 40
Typhoon 9200/9210/8600/8610 (GE Healthcare)	488 nm	526 SP
FluorImager (GE Healthcare)	488 nm	530 DF 30
Storm (GE Healthcare)	Blue (fluorescence mode)	
VDS-CL (GE Healthcare)	Transmission	UV Low
Ultracam/Gel Imager (Ultra-Lum)	UV	Yellow Filter (#990-0804-07)
Omega Systems (Ultra-Lum)	UV	520 nm
Polaroid Camera (Polaroid)	UV	SYBR® Safe Photographic Filter (S27100)
FOTO/Analyst Express/Investigator/Plus/ Luminary (FOTODYNE)	UV	Fluorescent Green (#60-2034)
FOTO/Analyst Minivisionary (FOTODYNE)	UV	Fluorescent Green (#62-4289)
FOTO/Analyst Apprentice (FOTODYNE)	UV	Fluorescent Green (#62-2535)
FOTO/Analyst Luminary (FOTODYNE)	UV	Fluorescent Green (#60-2056)
FCR-10 (Polaroid)	UV	#3-4218
FUJI FLA-3000 (FUJI Film)	473 nm	520LP
BioDocIt/AC1/EC3/BioSpectrum (UVP)	302 nm	SYBR® Green (#38-0219-01) or SYBR® Gold (#38-0221-01)
Gel Logic (Kodak)	UV	535 nm WB50
Syngene Instruments (Syngene)	UV	500–600 nm Shortpass filter

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat #	Product Name	Unit Size
S37102	Safe Imager™ blue-light transilluminator	each
S33100	SYBR® Safe DNA gel stain in 0.5X TBE	1 L
S33101	SYBR® Safe DNA gel stain in 0.5X TBE	4 L
S33102	SYBR® Safe DNA gel stain *10,000X concentrate in DMSO*	400 µL
S33110	SYBR® Safe DNA Gel Stain Starter Kit *with 1 L of SYBR® Safe DNA gel stain in 0.5X TBE (S33100) and one photographic filter (S37100)*	1 kit
S33111	SYBR® Safe DNA gel stain in 1X TAE	1 L
S33112	SYBR® Safe DNA gel stain in 1X TAE	4 L
S37100	SYBR® Safe photographic filter	each
G5218-01	E-Gel® 1.2% with SYBR® Safe	18 gels
G6206-01	E-Gel® 1.2% with SYBR® Safe Starter Kit	1 kit
G5218-02	E-Gel® 2.0% with SYBR® Safe	18 gels
G6206-02	E-Gel® 2.0% with SYBR® Safe Starter Kit	1 kit

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