E-Gel® 96 with SYBR® Safe

QUICK REFERENCE CARD

Catalog no. G7208-02

251001 Version B; 21 May 2007

Instructions are provided below for using E-Gel® 96 pre-cast agarose gels with SYBR® Safe DNA gel stain in the E-Base™ Integrated Power Supply. For more information and detailed instructions, refer to the E-Gel® Technical Guide available at www.invitrogen.com or contact Technical Support.

General Information

Kit Contents

Catalog no.	Product	Contents
G7208-02	E-Gel® 96 2% with SYBR® Safe	8 precast gels

Storage

Store at room temperature. Do not allow the temperature to drop below 4°C or rise above 40°C. Do not expose unnecessarily to light.

Product Description

E-Gel® 96 with SYBR® Safe combines the convenience of the bufferless, high-throughput E-Gel® 96 well pre-cast agarose gels with the safety of SYBR® Safe DNA gel stain. SYBR® Safe is a fluorescent DNA gel stain developed at Molecular Probes that exhibits sensitivity similar to that of ethidium bromide, while being considerably safer and environmentally friendly.

Advantages of SYBR® Safe

SYBR® Safe DNA gel stain offers the following advantages:

- SYBR® Safe DNA gel stain is not classified as hazardous waste under U.S. Federal regulations and meets the requirements of the Clean Water Act and the National Pollutant Discharge Elimination System regulations.
- SYBR® Safe DNA gel stain does not cause mutations, chromosomal aberrations, or transformations in appropriate mammalian test systems, in contrast to ethidium bromide which is a strong mutagen.
- A single oral administration of SYBR® Safe DNA gel stain produces no signs of mortality or toxicity at a limit dose of 5000 mg/kg.
- Visualizing E-Gel® with SYBR® Safe using blue light transilluminators dramatically reduces DNA damage that lowers cloning efficiency.

View studies documenting the safety of SYBR® Safe in the SYBR® Safe White Paper document, available from

http://probes.invitrogen.com/media/publications/494.pdf

Advantages of E-Gel® 96 Gels

E-Gel® 96 Gels for electrophoresis of DNA offer the following advantages:

- High-throughput, robot-compatible, and ready to put into action.
- Provides fast, safe, consistent, high-resolution electrophoresis.
- Eliminates the need to prepare agarose gels and buffers, and to stain gels.

Disposal

SYBR® Safe DNA gel stain shows 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. Many institutions and municipalities have approved safe disposal of SYBR® Safe into their waste water systems. As disposal regulations vary, please contact your safety office or local municipality for appropriate SYBR® Safe disposal in your community.

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Procedure

General Guidelines

For best results with E-Gel® 96 with SYBR® Safe follow these guidelines:

- Use the recommended DNA amount and sample volume to prepare samples.
- Load 500 ng of molecular weight markers (we recommend E-Gel® Low Range Quantitative DNA Ladder, Cat. no. 12373-031).
- Keep all sample volumes uniform and load deionized water in empty wells.
- Load gel within 30 minutes after opening the pouch; run the gel within 15 minutes after loading.
- Dilute high salt samples 2- to 20-fold before loading.
- While yielding similar sensitivities to ethidium bromide, it is worth noting that SYBR® Safe is somewhat dimmer yet with a lower background than ethidium bromide. Consider viewing the gels in a darkened room.

Prepare Samples

- Use 20-100 ng DNA per band for samples containing one unique band, though 5 ng or 200 ng per band will still yield consistently good results. For samples containing multiple bands, load up to 500 ng per lane.
- Prepare DNA samples in a **total sample volume of 20 µl** in deionized water or loading buffer (recommended final concentration: 10 mM Tris-HCl; pH7.5; 1 mM EDTA, 0.005% bromophenol blue; and 0.005% xylene cyanol FF).
- Dilute **high salt samples** (samples with >50 mM NaCl, >100 mM KCl, >10 mM acetate ions, >10 mM EDTA), 2- to 20-fold in deionized water, TE, or loading buffer in final volume of 20 µl.

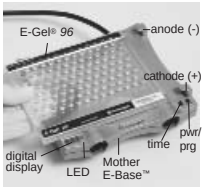
Setting Up E-Base™

The recommended program for E-Gel® 96 with SYBR® Safe gel is EG and the run time is 12 minutes. Alternatively, use the EP program with a 6 minute run time, though this may result in a slight reduction of run quality.

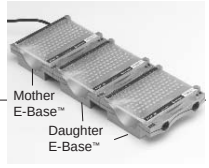
1. Plug Mother E-Base™ into an electrical outlet. Connect Daughter E-Base™ to a Mother E-Base™ or another Daughter E-Base™ connected to a Mother E-Base™.
2. Press and release the **pwr/prg** (power/program) button on the base to select program EG.

Note: E-Gel® 96 with SYBR® Safe gels are compatible with the E-Gel® *96* base available previously from Invitrogen; refer to the E-Gel® Technical Guide.

Mother E-Base™



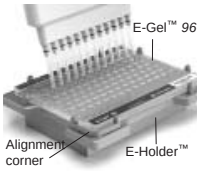
Daughter E-Base™



Using E-Holder™ Platform (Optional)

1. The E-Holder™ is designed to hold E-Gel® *96* gels during robotic loading and allows loading multiple gels on a robotic platform.
   Note: The E-Holder™ is not a power supply and cannot be used to run E-Gel® 96 with SYBR® Safe gels.
2. Place the E-Holder™ on the robotic platform.
3. Remove gel from the package and remove comb from the gel.
4. Place E-Gel® 96 cassette on the E-Holder™. Align the bottom left end of the cassette in the lower left alignment corner of the E-Holder™.
5. Set up your robotic system to load samples into the gel placed on the E-Holder™. Program robotic system to load the samples ~5 minutes prior to the completion of the previous run to ensure that loaded gel on E-Holder™ will be placed on an E-Base™ within the recommended time of 15 minutes.

E-Holder™



Procedure, continued

Loading and Running Gel

1. Remove gel from the package and remove plastic comb from the gel.
2. Slide gel into the two electrode connections on the Mother or Daughter E-Base™. If gel is properly inserted, a fan in the base begins to run, a red light illuminates, and digital display shows 12 minutes.

Loading Gels

3. Load 20 µl prepared DNA sample into the well. Keep all sample volumes uniform. Load samples manually, with a multichannel pipettor, or use robotic loading devices (8-, 12-, 96-tip).
   Note: To ensure proper sample loading with robotic loading device, align the robotic tip assembly (see E-Gel® Technical Guide for details).
4. Load the E-Gel® Low Range Quantitative DNA Ladder in the marker wells. Be sure the marker salt concentration is similar to that of adjacent samples.
5. Load 20 µl sample buffer containing the same salt concentration as the sample into any empty wells.
6. Press and release **pwr/prg** button on the E-Base™ to begin electrophoresis. The red light changes to green.
7. At the end of the run (signaled with a flashing red light and rapid beeping),press and release the **pwr/prg** button to stop the beeping.

Visualization

Bound to nucleic acids, SYBR® Safe DNA gel stain has fluorescence excitation maxima at 280 and 502 nm, and an emission maximum at 530 nm (Fig. 1). View E-Gel® with SYBR® Safe these devices:

- Blue-light transilluminator, such as Invitrogen's Safe Imager™ Blue Light Transilluminator, S3710. Use the Safe Imager™ amber filter unit or viewing glasses provided with the device to view the gel.
- Standard 300 nm UV transilluminator.
- Imaging systems such as laser based scanners equipped with an excitation source in the UV range or between 470-530 nm

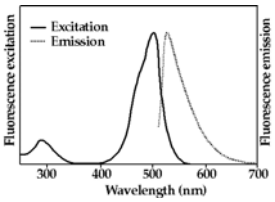


Figure 1. Normalized fluorescence excitation and emission spectra of SYBR® Safe DNA gel stain, determined in the presence of DNA.

Notes: - Avoid overexposure of skin and eyes to UV light and eyes to blue light.
- The signal over a UV transilluminator is less intense and less sensitive than that over a Safe Imager™ Blue Light Transilluminator.

Filters

For photographing gels a filter is needed, such as SYBR® Safe photographic filter (S37100), Molecular Probes' SYPRO® photographic filter (S6656) or Kodak® Wratten #9 filter. A long pass ethidium bromide filter that transmits all light above 500 nm also works; other ethidium bromide filters may not.

Refer to the **E-Gel® Technical Guide** to determine the optimal filter sets to use, or contact the instrument manufacturer for advice.

Imaging

- Photograph E-Gel® with SYBR® Safe using Polaroid® 667 black-and-white print film, or image using a CCD camera or a laser-based scanner.
- Analyze digital images, and arrange lanes within the image, using the E-Editor™ 2.0 software available for FREE at www.invitrogen.com/egels.
- Slightly longer exposure time, or higher gain setting may be necessary compared to Ethidium Bromide stained gels.

Troubleshooting

Problem	Cause	Solution
No current	Cassette improperly inserted or is defective	Remove the gel cassette and re-insert the cassette correctly, or try using a fresh cassette.
	Only Daughter E-Base™ used	Daughter E-Base™ does not have an electrical plug to connect to an outlet. Always use it with a Mother E-Base™.
Poor resolution or smearing of bands	Sample overloaded	Do not load more than 100 ng of DNA per band.
	High salt samples	Dilute your samples 2- to 20-fold
	Sample not loaded properly or low sample volume loaded	Do not introduce bubbles while loading samples. For proper resolution, keep all sample volumes uniform and load water into empty wells. Use Two-Step Loading (see E-Gel® Technical Guide for details).
Sample leaking from wells	Wells damaged during comb removal	Be sure to remove the comb gently without damaging the wells.
	Sample is overloaded	Load the recommended sample volume per well. Use Two-Step Loading (see **E-Gel® Technical Guide** for details).
Weak signal	SYBR® Safe is dimmer but has lower background than ethidium bromide.	Consider viewing the gels in a darkened room.
High background, suboptimal, or no image	No filters or wrong filter set.	Refer to **E-Gel® Technical Guide** to determine the optimal filter sets to use, or contact the instrument manufacturer for advice.
	Photographic settings not optimal.	Optimize settings of your system for E-Gel® with SYBR® Safe empirically. You may need to increase the exposure time or gain setting.
Stripes visible on image	No IR coating on camera lense.	Use IR blocking filter or emission filter with IR coating.

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Limited Use Label License No. 188: SYBR® Safe Nucleic acid Stain

This product is provided under an agreement only for the use in staining nucleic acids in gels.

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