E-Gel™ Agarose Gels with SYBR Safe™

Catalog nos. G5218-01, G5218-02, G6206-01, G6206-02
Pub. Part No. 25-0862 **Pub. No.** MAN0003477 **Revision** A.0

General information

E-Gel™ with SYBR Safe™ gels combine the convenience of the bufferless E-Gel™ 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.

Instructions are provided below for using E-Gel™ pre-cast agarose gels with SYBR Safe™ DNA gel stain in the E-Gel™ PowerBase™ v.4. For more information and detailed instructions, refer to the E-Gel™ Technical Guide available at **thermofisher.com** or contact Technical Support.

Advantages of SYBR Safe™ DNA Gel Stain

- 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™ agarose gels using blue light transilluminators dramatically reduces DNA damage that lowers cloning efficiency.

Advantages of E-Gel™ Agarose Gels

Using E-Gel™ agarose gels for electrophoresis of DNA samples offer the following advantages:

- Provides fast, safe, consistent, high-resolution electrophoresis.
- Eliminates the need to prepare agarose gels and buffers, and to stain gels.

Kit contents

Product	G5218-01	G5218-02	G6206-01	G6206-02
E-Gel™ with SYBR Safe™ 1.2% agarose	18 precast gels	—	6 precast gels	—
E-Gel™ with SYBR Safe™ 2% agarose	—	18 precast gels	—	6 precast gels
E-Gel™ PowerBase™	—	—	1	1

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.

Procedure

General guidelines

For best results with E-Gel™ agarose gels, follow these guidelines:

- Prepare samples with recommended DNA amount and sample volume.
- Load 500–700 ng of molecular weight markers in an appropriate volume.
- Keep all sample volumes uniform. Load deionized water in empty wells.
- Load gel within 15 min of opening the pouch. Run the gel immediately after loading.
- Dilute samples that contain high salt concentration buffers (certain restriction enzyme and PCR buffers) 2- to 20-fold before loading (refer to the E-Gel™ Technical Guide for details).

Prepare samples

For best results load 20–100 ng DNA per 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 for E-Gel™ 1.2% with SYBR Safe™ gels and up to 700 ng for E-Gel™ 2% with SYBR Safe™ gels.

The total sample volume for One-Step loading (below) is 20 µL.

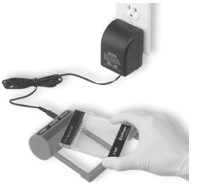
You may prepare DNA samples in deionized water or loading buffer.

Load samples

Step 1

1. Remove E-Gel™ agarose gel from package and remove the comb from the cassette.
2. Insert the gel into the base, starting from the right edge of the cassette. The Invitrogen logo should be located at the bottom of the base. Press firmly at the top and bottom to seat the gel in the base. A steady, red light illuminates on the base if the gel is correctly inserted.

Steps 2, 3



3. Plug the E-Gel™ PowerBase™ v.4 into an electrical outlet using the adaptor plug on the base.

   Note: Instructions for using an E-Gel™ agarose gel with the E-Gel™ iBase™ device is included in the E-Gel™ Technical Guide.

4. Load 20 µl of prepared sample into each well.
5. Load appropriate DNA molecular weight markers.
6. Load 20 µl of water into any remaining empty wells.

Steps 4–6

Two-step loading method

If the gel produces fuzzy or indistinct bands, use Two-Step loading for steps 4–6 (above).

1. Load 10 µL of deionized water into each well.
2. Load 10 µL of prepared sample per sample well (10% glycerol may be added).
3. Load 10 µL of prepared DNA molecular weight marker (10% glycerol may be added).
4. Load 10 µL of water into any remaining empty wells.

Run conditions

1. Press and release the 30-minute button on the E-Gel™ PowerBase™ device to begin electrophoresis. At the end of each run, the current automatically shuts off and the power base signals the end of the run with a flashing red light and rapid beeping.
2. Press and release either button to stop the beeping. Remove the gel cassette and analyze your results.

Visualization

SYBR Safe™ DNA gel stain has a fluorescence excitation maxima at 280 and 502 nm, and an emission maximum at 530 nm (Fig. 1) when bound to nucleic acids. View E-Gel™ with SYBR Safe™ using:

- Blue-light transilluminators, such as the Safe Imager™ Blue Light Transilluminator, (Cat. no. G6600), or the E-Gel™ Safe Imager™ Real-Time Transilluminator (Cat. no. G6500). Use the Safe Imager™ amber filter unit or viewing glasses provided with the device to view the gel and to avoid overexposure of eyes to blue light.
- 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.

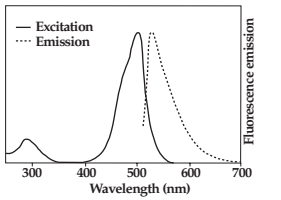


Figure 1. Normalized fluorescence excitation and emission spectra of SYBR Safe™ DNA gel stain, determined in the presence of DNA.

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™ gels using a CCD camera or a laser-based scanner. 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. As a result a slightly longer exposure time, or higher gain setting may be necessary.

Expected results

Figure 2: DNA samples (10–700 ng per band) on E-Gel™ 2% with SYBR Safe™ gels, photographed using the SYBR Safe™ photographic filter (S37100) with blue light transillumination (Figure 2A) or UV transillumination (Figure 2B).

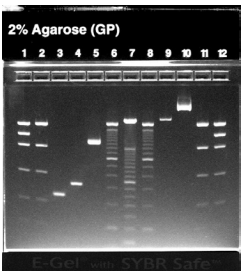


Figure 2A: Blue light

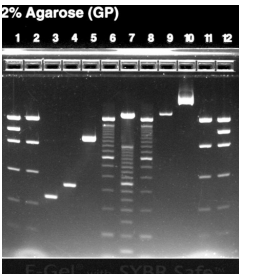


Figure 2B: UV

Troubleshooting

Observation	Cause	Solution
No current	Cassette improperly inserted or is defective	Remove the gel cassette and re-insert the cassette correctly. Use a fresh cassette.
Poor resolution or smearing of bands	Sample overloaded	Do not load more than 200 ng of DNA per band in a volume of 20 µL.
	High salt samples	Dilute your samples 2- to 20-fold as described in the E-Gel™ Technical Guide.
	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 method (see E-Gel™ Technical Guide).
Melted gel	Increased current due to longer run times	Do not run the gel longer than 40 minutes.
Sample leaking from wells	Wells damaged during comb removal	Be sure to remove the comb gently without damaging the wells.
	Sample overloaded	Load 20 µL of sample per well. Use the Two-Step Loading method (see the E-Gel™ Technical Guide).
High background, suboptimal, or no image	No filters or wrong filter set.	See 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.

Disposal

SYBR Safe™ DNA gel stain shows no or very low mutagenic activity, and is not classified as hazardous waste under U.S. Federal regulations. Many institutions have approved safe disposal of SYBR Safe™ gel stain into their waste water systems. As disposal regulations vary, contact your safety office or local municipality for appropriate SYBR Safe™ gel stain disposal in your community.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Corporate entity: Life Technologies | Carlsbad, CA 92008 USA | Toll Free in USA 1.800.955.6288

For support visit thermofisher.com/support

1 November 2015

ThermoFisher SCIENTIFIC