E-Gel® EX Agarose Gels

Catalog nos. G6511ST, G6512ST, G4020-01, G4020-02, G4010-01, G4010-02, G4010-04

Part no. 25-1038 Rev. Date: 6 August 2009

QUICK REFERENCE CARD

E-Gel® EX agarose gels are pre-cast 1%, 2%, and 4% agarose gels, for use with the E-Gel® iBase™ Power System. E-Gel® EX gels have 11 wells, and a novel openable format. A proprietary fluorescent nucleic acid stain in the gel allows detection down to 1 ng/band of DNA when visualized by blue light transilluminator (excitation at 490 nm/emission at 522 nm). For more information and detailed instructions, refer to the E-Gel® Technical Guide available at www.invitrogen.com or contact Technical Support.

General Guidelines

- Store gels at room temperature
- For samples or DNA ladders in high salt buffer, dilute 2- to 20-fold before loading
- Load 100–250 ng of DNA ladder diluted in an appropriate volume
- Prepare DNA samples and markers in deionized water, or the E-Gel® Sample Loading Buffer (Invitrogen Catalog no. 10482-055)
- Keep sample volumes uniform and load deionized water into empty wells
- Load gel within 15 min of opening the pouch; run within 1 min of loading sample
- Use a blue-light transilluminator (e.g., E-Gel® Safe Imager™) to visualize DNA (observe safety instructions described in manual)
- To prepare and run RNA samples, refer to the E-Gel® Technical Guide

Sample Preparation

- **Use a total sample volume of 20 µl for each well**
- Adjust the amount of DNA sample according to the number of bands being separated

Agarose Gel %	Single DNA Band	Multiple DNA Bands	Optimal Sample Amount	Maximum Sample Amount
1%	1–100 ng	1–50 ng/band	3–25 ng	250 ng
2%	1–300 ng	1–100 ng/band	5–150 ng	500 ng
4%	1–300 ng	1–100 ng/band	5–200 ng	500 ng

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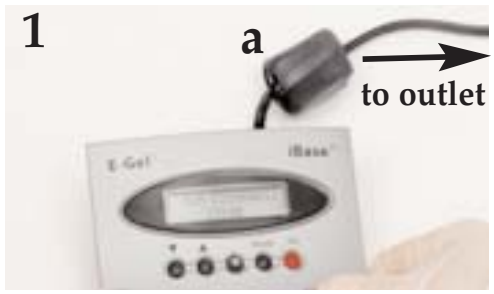
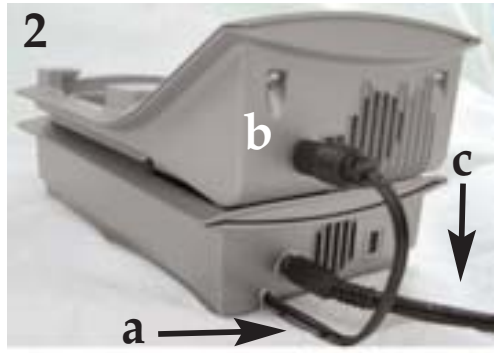
Contact Information for Other Countries:
See our website **www.invitrogen.com**

One-Step Loading of E-Gel® EX Agarose Gel

1. If using the iBase™ without an E-Gel® Safe Imager™, connect the cord with the transformer (a) to the power inlet of the iBase™, and plug the other end into an electrical outlet. Verify that your iBase™ firmware has the following programs to run your gels:
   "E-Gel® EX 1–2%" program for E-Gel® EX 1% and 2% gels
   "E-Gel® EX 4%"program for 4% E-Gel® EX gels
   See **Downloading Upgrades** if programs are not present.



2. If using the iBase™ with an E-Gel® Safe Imager™, place the iBase™ on top of the Safe Imager™, and plug the short cord (a) from the Safe Imager™ into the power inlet of the iBase™ (b). Plug the connector of the power cord with the transformer into the Safe Imager™ (c), and connect the other end of the power cord to an electrical outlet.



3. Remove the gel from the package and gently remove the comb from the E-Gel® EX cassette.

4. Insert the gel into the E-Gel® iBase™ Power System, starting from the right edge. Press firmly at the top and bottom to seat the gel in the base. A steady light illuminates on the iBase™ if the cassette is correctly inserted.

5. Load gel **without** pre-running as follows:
   - 20 µl sample into each well
   - 20 µl appropriately diluted DNA ladder
   - 20 µl deionized water into any empty wells

Note: Using DNA ladders with EDTA concentrations >0.25 mM result in low resolution and limited separation. For best results, the following DNA ladders from Invitrogen are recommended:

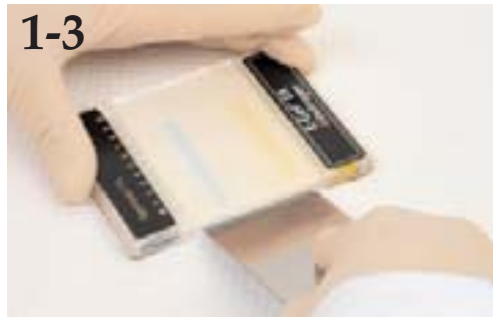
Gel Type	Product	Catalog no.
1% and 2% E-Gel® EX gels	E-Gel® 1Kb Plus DNA Ladder	10488-090
4% E-Gel® EX gels	E-Gel® 25bp DNA Ladder	10488-095
	E-Gel® 50bp DNA Ladder	10488-099

Run Conditions

Important: Do not pre-run E-Gel® EX gels.

1. Place the amber filter over the E-Gel® iBase™.
2. Select the program, and set the run time on the iBase™ according to the percentage of the gel being run:
   - "E-Gel® EX 1–2%" (program 7) 10 minutes
   - "E-Gel® EX 4%" (program 8) 15 minutes
3. Press the **Go** button on the iBase™. The red light turns to a green light, indicating the start of the run.
4. The run stops automatically after the the programmed time has elapsed. The end of the run is signaled by a flashing red light and rapid beeping. The LCD displays "Run Complete Press Go".

Opening the E-Gel® EX Cassette



1. Place the cassette on a bench with the wells facing up.
2. Insert the sharp edge of the gel knife in the groove between the cassette halves, and lever the knife up and down. Repeat for every edge of the cassette.
3. Open the cassette and excise the band.
4. Dispose of the used gel as hazardous waste.

Visualization and Imaging

- View E-Gel® EX gels with an E-Gel® Safe Imager™ or other blue light transilluminator
- Always use an amber filter, or amber viewing goggles
- For imaging with a laser based scanner, verify the system has an excitation source compatible with the proprietary dye
- E-Gel® EX gels can be viewed by UV illumination, but sensitivity will be reduced
- To photograph gels with a CCD camera, a photographic filter is required e.g. SYBR Safe® filter (Invitrogen Catalog no. S37100) or Molecular Probes SYPRO® filter (Invitrogen Catalog no. S6656)
- Refer to the **E-Gel® Technical Guide** to determine the optimal filter sets to use, or contact the instrument manufacturer for advice

Downloading Upgrades

If your E-Gel® iBase™ Power System has an older firmware version lacking the "E-Gel® EX 1–2%" and the "E-Gel® EX 4%" programs, download new iBase™ firmware versions at www.invitrogen.com/ibase. Follow the instructions on the page to upgrade your device.

Troubleshooting

Problem	**Cause**	**Solution**
No Current	Cassette improperly inserted or defective	Remove and re-insert cassette or try using new cassette.
Poor resolution, smeared bands, poor migration	Sample overloaded	Use correct amount of sample as described in **Sample Preparation.** Dilute sample with TE.
	High salt	Dilute samples as directed in the E-Gel® Technical Guide.
	Sample improperly loaded or sample volume too low	**Do not** introduce bubbles when loading. Keep all volumes uniform and load water into empty wells.
RNA sample cannot be seen	Inhibition of visualizaton by heat and denaturing agent	Wait 10–15 min for gel to cool before visualization.
Melted gel	Run time too long leading to increased current	**Do not** run 1% or 2% gels longer than 15 min, or 4% gels longer than 20 min.
Leaking samples	Wells damaged during comb removal	Remove comb gently without damaging the wells.
	Sample volume too large	Use recommended volume in each well. Use two-step loading method described in the E-Gel® Technical Guide.
High background, sub-optimal or no image	No filter or wrong filter set	Refer to E-Gel® Technical Guide or instrument manufacturer for optimal filter set.
	Photographic settings not optimal	Determine optimal settings empirically by adjusting exposure time, gain, etc.

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