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E-Gel® SizeSelect™ Agarose Gels

Cat. nos. G661002, G6612ST, G6612STEU, G6612STUK

Publication Part no. 25-1043 MAN0006812 **Rev. Date** 11 April 2012

E-Gel® SizeSelect™ 2% agarose gels provide a novel way to purify DNA. The gels have a top row of wells for loading sample, and a bottom row of wells to retrieve DNA bands of interest. The gels contain a proprietary nucleic acid stain that allows detection down to 1.5 ng/band of DNA. The following instructions describe using E-Gel® SizeSelect™ gels with the E-Gel® iBase™ Power System.

For detailed instructions, refer to the E-Gel® Technical Guide available at www.lifetechnologies.com.

General Guidelines

- Store gels at room temperature.
- Prepare DNA samples and markers in deionized water or 1× TE.
- Dilute samples with high salt concentration 2- to 20-fold before loading.
- Do not use loading buffer with tracking dye to avoid masking bands.
- Load gel within 15 min of opening pouch; run within 1 min of loading.
- Keep sample volumes uniform. Load deionized water into empty wells.
- Load 100–250 ng of DNA ladder in an appropriate volume to lane M.
- Visualize DNA by blue-light transillumination (ex/em at 490/522 nm).

Prepare Sample

1. Use a total sample volume of 20–25 µL for each well.
2. Load up to 700 ng of total sample per well (1.5–300 ng per band).
3. Collect high quantities of DNA in two separate fractions.

For research use only. Not for human or animal therapeutic or diagnostic use.

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Run Time Estimation

Refer to the Run Time Table to estimate the run time for your DNA fragment to reach the reference line, and then from the reference line to reach the collection well. Monitor your gel during the run. If the amount of DNA is low, the band may not be visible. Viewing the gel in a darkened room may improve visualization.

The 50 bp ladder (Cat. no. 10416-014) can be run as a size reference marker.

Collect the band of interest when the two bands in the 50 bp ladder that bracket the band of interest in size are just beginning to enter (larger marker) and exit (smaller marker) the collection well in the marker lane.

Band Size	Run Time to Reference Line*	Time from Reference Line to Collection Well*
50 bp	9–10.5 min	0.5–1.5 min
100 bp	9.5–11.5 min	1–1.5 min
150 bp	10.5–13.5 min	1–1.5 min
200 bp	11–14.5 min	1–2 min
300 bp	13–16 min	1–2 min
400 bp	15–19.5 min	1.5–2.5 min
500 bp	16.5–21.5 min	1.5–2.5 min
650 bp	18.5–25.5 min	1.5–2.5 min
800 bp	19–28 min	2–2.5 min
1000 bp	21–30.5 min	3–3.5 min

*Indicated run times are estimates. Some bands in different wells may migrate differently due to variations in DNA quantity, fragment size, and salt content.

Downloading Upgrades

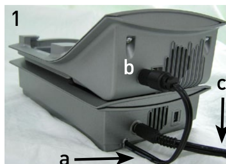
For users of E-Gel® iBase™ Power System with older firmware versions without the "SizeSelect 2%" program, download new iBase™ firmware versions at www.lifetechnologies.com/ibase. Follow the instructions on the page to upgrade your device.

Troubleshooting

Observation	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**.
	High salt	Dilute samples as directed in E-Gel® Technical Guide.
	Sample improperly loaded or volume too low	**Do not** introduce bubbles when loading. Keep all volumes uniform and load water into empty wells.
Melted gel	Run time too long leading to increased current	**Do not** run gels longer than 30 minutes.
	Incorrect program selected	Run gel using "**SizeSelect 2%**" program.
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.
Stripes visible on image	No IR coating on camera lens	Use IR blocking filter or emission filter with IR coating.
Band of interest below collection well	Run time too long	Use Reverse program as described in the E-Gel® Technical Guide to run band back into collection well.
Low volume in collection well	Well not refilled prior to collection	Fill the second row of wells with sterile water prior to running your band of interest into the wells.
Low yield, bands smeared	Excess quantity of DNA	Collect DNA from the well in two or more fractions (refill with water after each collection). Load the recommended amount of DNA.
Low yield, bands not visible	Low quantity of DNA	Load the recommended amount of DNA. View gel in darkened room or use 50 bp ladder as reference marker. Refer to **Run Time Table** to determine when to collect the sample.

One-Step Loading of E-Gel® SizeSelect™ Agarose Gel

1. Place the iBase™ on top of the Safe Imager™, 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. Verify that the iBase™ has the "SizeSelect 2%" program. If not, see **Downloading Upgrades**.

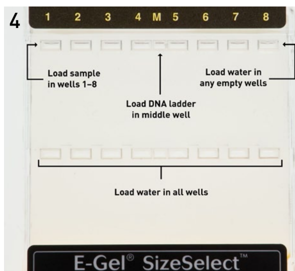


2. Remove the gel from the package and gently remove the comb from the E-Gel® cassette.

3. Insert the gel into the E-Gel® iBase™ Power System right edge first. Press firmly at the left edge to seat the gel in the base. A steady light illuminates on the iBase™ if the cassette is correctly inserted.

4. Load gel **without** pre-running as follows:

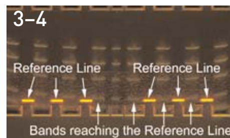
- 20–25 µL sample into each well of top row
- 5–10 µL appropriately diluted DNA ladder into middle well (lane M)
- 25 µL deionized water into all empty wells (top row)
- 25 µL deionized water into all large bottom (collection) wells and 5–10 µL for lane M of the bottom row

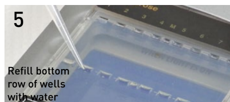


Run Conditions

Important: Do not pre-run E-Gel® SizeSelect gel

1. Place the amber filter over the E-Gel® iBase™.
2. Select "**SizeSelect 2%**" program (program 8) and set time to the **Run Time to Reference Line** as listed in the **Run Time Estimation Table** according to band size. Press the **Go** button on the iBase™. The red light turns to a green light indicating the start of the run.
3. Run your band of interest to the reference line. Monitor the run periodically, and press the **Go** button to stop the run when the band reaches the reference line. Proceed to Step 5.
4. The end of the run is signaled with a flashing red light and rapid beeping. If the band has not reached the reference line, run the gel longer until the band reaches the line.
5. When the band reaches the reference line, refill the collection wells to 25 µL with sterile water. The refill volume may vary between wells. **Do not overfill**.
6. Enter the appropriate time listed under **Run Time from Reference Line to Collection Well** from the **Run Time Estimation Table** for your band. Press **Go** to run the gel. Monitor the run carefully. As the run ends, the band of interest may be seen migrating into the collection well.





Run Conditions, continued

7. Collect DNA from the wells using a pipette. Make sure not to pierce the bottom of the well. Proceed to your application using the collected DNA. If the band of interest has overshot the collection well, use the "REVERSE E-Gel" program to run the band back into the collection well.
8. Additional DNA bands can be collected from the same well(s). Be sure to refill the collection wells with more water, as water is lost during the run.
9. Dispose of used gels as hazardous waste.

Imaging and Quantitation

- Visualize gels with the E-Gel® Safe Imager™ (Cat. no. G6500), or other blue-light transilluminator.
- Recovered DNA can be assessed using the Qubit® fluorometer (Cat. no. Q32868), or by gel electrophoresis.
- qPCR is recommended for accurate quantitation of next generation sequencing libraries recovered from SizeSelect™ gels.
- Recovered samples are not compatible with 280 nm measurements without first performing buffer exchange.
- Refer to the E-Gel® Technical Guide or contact Technical Support for additonal information on imaging, filters, and quantitation.

NOTICE TO PURCHASER: PLEASE REFER TO THE E-GEL® TECHNICAL GUIDE FOR LIMITED LABEL LICENSE OR DISCLAIMER INFORMATION.

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