E-Gel™ SizeSelect™ II Agarose Gel

invitrogen

QUICK REFERENCE Pub. No. MAN0017341 Rev. B.0

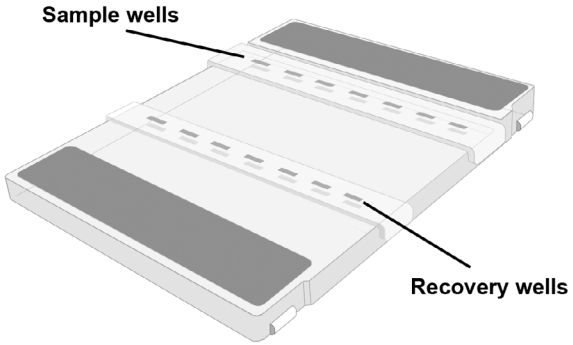
Contents

Catalog Number G661012

Components	Amount
E-Gel™ SizeSelect™ II Agarose Gel, 2%	10 gels
10X Sample Loading Buffer	500 µL

Product description

- The Invitrogen™ E-Gel™ SizeSelect™ II Agarose Gels provide a fast and convenient method for DNA fragment library size selection as part of NGS library preparation workflows.
- E-Gel™ SizeSelect II Agarose Gels are specifically designed for use with the E-Gel™ Power Snap Electrophoresis Device (Cat. No. G8100, G8300).



E-Gel™ SizeSelect II Agarose Gel

Online resources

- Visit our product pages for protocols, safety, and additional product information.
- Go online to view related E-Gel™ products.
- For support, visit thermofisher.com/support.

Required materials

- E-Gel™ Sizing DNA Ladder (Cat. No. 10488100)
- UltraPure™ DNase/RNase-Free Distilled Water (Cat. No. 10977023)
- 10X Sample Loading Buffer (included with E-Gel™ SizeSelect™ II Agarose Gels)
- E-Gel™ Power Snap Electrophoresis Device (Cat. No. G8100)
- (*Optional*) E-Gel™ Power Snap Camera (Cat. No. G8200)
- Safe Imager™ viewing glasses (Cat. No. S37103; included with G8100)

Important guidelines

- Do not exceed 500 ng of total DNA per one sample lane or 500 ng DNA for a single band. Do not exceed 1 µg for sheared DNA.
- Samples containing ≥50 mM NaCl, 100 mM KCl, 10 mM acetate ions, or 10 mM EDTA (i.e., certain restriction enzyme and PCR buffers) will cause loss of resolution on E-Gel™ agarose gels. Dilute samples containing high salt levels 2- to 5-fold to obtain the best results.
- Load the E-Gel™ SizeSelect II agarose gel within 15 minutes after opening the pouch, and run the gel within 1 minute after loading samples.
- Always wear Safe Imager™ viewing glasses when working with the E-Gel™ Power Snap Electrophoresis Device cover open.

Troubleshooting

For detailed troubleshooting instructions see the E-Gel™ Power Snap Electrophoresis System User Guide at thermofisher.com or contact Technical Support.

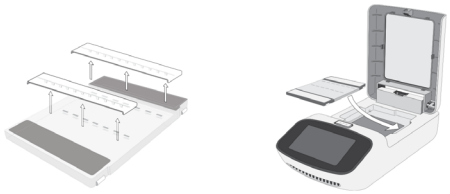
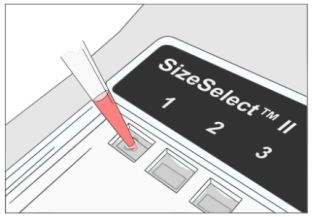
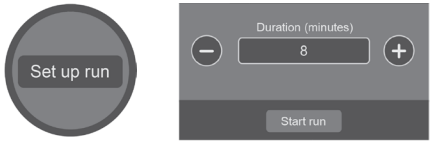
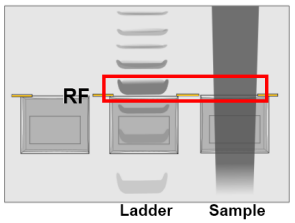
Limited product warranty and licensing information

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

For Research Use Only. Not for use in diagnostic procedures.

ThermoFisher SCIENTIFIC

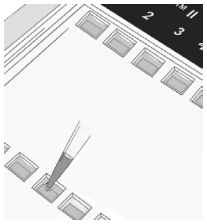
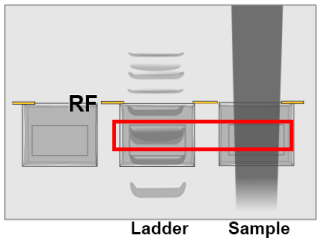
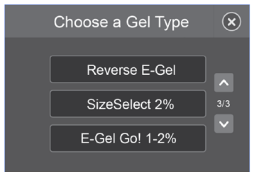
DNA electrophoresis protocol

	Step		Action
5–10 min	1 	**Prepare samples**	Prepare samples in a total volume of 25 µL. Scale volumes proportionally for lower volumes. Component: Sample — Volume: 22.5 µL Component: 10X Sample Loading Buffer — Volume: 2.5 µL Important! See **Important guidelines** for sample amount limitations and samples containing high salt content.
	2 	**Prepare gel**	a. Remove the gel from the package and gently remove the combs. Do not allow the combs to bend or create suction in the wells during removal. b. Insert gel cassette into the E-Gel™ Power Snap Electrophoresis Device, starting from the right edge. Press down on the left side of the cassette to secure the cassette.
	3 	**Load samples**	a. Fill all wells of both rows with 50 µL of deionized water. b. Load 25 µL of sample with 1X Sample Loading Buffer into wells from the bottom up. Do not damage the gel or introduce bubbles into the wells. c. Load 25 µL of 1X E-Gel™ Sizing DNA Ladder into a marker well.
12–40 min	4 	**Run the gel**	a. Set up run by selecting the **SizeSelect 2%** protocol on the E-Gel™ Power Snap Electrophoresis Device. b. Adjust protocol time according to expected migration time of your target fragment to the reference line (see **Guidelines for estimating run time**). c. Press **Start run** to start the gel protocol.
	5 	**Check status**	a. Check gel status by activating the Back light button. Monitor the gel during the run to avoid target fragment missing the recovery well. b. Pause the gel when the reference band of the DNA ladder reaches the reference line (RF) on the second row of recovery wells. c. Proceed to **DNA collection protocol**.

DNA collection protocol

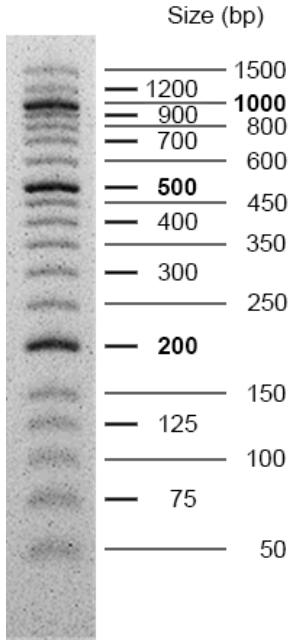
Important! Wear Safe Imager™ viewing glasses before starting the DNA collection protocol.

Reduce ambient light or work in dark room for better visibility.

	Step		Action
5 min	6  	**Prepare wells**	a. Open the filter lid of the E-Gel™ Power Snap Electrophoresis Device, and activate the Back light. b. Carefully remove all liquid from the recovery wells. c. Load 50 µL of nuclease-free water to all recovery wells. Do not allow water to spill over the edge of the wells.
	7 	**Collect DNA fragment**	a. Resume the run and carefully observe as the reference band of the DNA ladder reaches the position described in the **NGS library size selection guidelines** for the desired target library peak. b. Stop the gel and recover the sample with a pipette. Avoid piercing the agarose during collection. c. Proceed with downstream NGS workflow.
	8  	**Reverse run (*Optional*)**	a. Stop the run and switch to the **Reverse E-Gel** protocol if the band of interest passes the collection well. b. Press **Set up run** and select the **Reverse E-Gel** protocol c. Press **Start run**. d. Carefully observe as the band of interest enters the recovery well and collect DNA fragment.

Guidelines for estimating run time

- Refer to the E-Gel™ Sizing Ladder migration pattern table to estimate target DNA run time to the reference line.
- The E-Gel™ DNA Sizing Ladder is also used as a size reference marker. See **NGS library size selection guidelines** to estimate run time from the reference line to the collection well.
- The run times indicated in the table are estimates. Monitor your gel in real time during the run to ensure the sample does not pass the recovery well.
- Identically sized bands in different wells may migrate differently.
- DNA fragment size, amount, and salt content can affect migration rates.

Ladder	Fragment size	DNA amount (per 25 µL)	Migration time to reference line
	1,500 bp	1.5 ng	~19.5 min
	1,200 bp	1.5 ng	~18.5 min
	1,000 bp	6.0 ng	~17.5 min
	900 bp	2.0 ng	~17 min
	800 bp	2.0 ng	~16.5 min
	700 bp	2.0 ng	~16 min
	600 bp	2.0 ng	~15.5 min
	500 bp	6.0 ng	~14.5 min
	450 bp	2.0 ng	~14 min
	400 bp	2.0 ng	~13.5 min
	350 bp	2.0 ng	~13 min
	300 bp	2.0 ng	~12.5 min
	250 bp	2.0 ng	~11.5 min
	200 bp	6.0 ng	~11 min
	150 bp	2.0 ng	~10 min
	125 bp	2.0 ng	~9.5 min
	100 bp	2.0 ng	~9 min
	75 bp	2.5 ng	~8.5 min
	50 bp	2.5 ng	~8 min

NGS library size selection guidelines

Sequencing system	Library size	Target library peak	Run time to reference line	Input sample amount	Stop the run and collect your sample when...	Schematic view
Ion PGM™ System	400-base-read	480 bp	14–20 min	500 ng	500-bp band is at the **top** of the **exposed agarose area**.	 500 bp
				50–100 ng	500-bp band has **just entered the top edge** of the **collection well**.	 500 bp
	300-base-read	390 bp	13–16 min	500 ng	400-bp band is at the **middle** of the **exposed agarose area**.	 400 bp
				50–100 ng	500-bp band is at the **top** of the **exposed agarose area**.	 500 bp
	200-base-read	330 bp	12–14 min	500 ng	350-bp band at the **top** of the **exposed agarose area**.	 350 bp
				50–100 ng	350-bp band has **just completely entered the top edge** of the **collection well**.	 350 bp
	100-base-read	200 bp	11–12.5 min	500 ng	200-bp band is in the **middle** of the **collection well**.	 200 bp
				50–100 ng	200-bp band is in the **middle** of the **collection well**.	 200 bp
Ion Proton™ System	200-base-read	270 bp	12–14 min	500 ng	300-bp band is at the **top** of the **exposed agarose area**.	 300 bp
				50–100 ng	300-bp band is at the **middle** of the **exposed agarose area**.	 300 bp
	150-base-read	220 bp	11–14.5 min	500 ng	250-bp band is at the **middle** of the **exposed agarose area**.	 250 bp
				50–100 ng	250-bp band is at the **middle** of the **exposed agarose area**.	 250 bp