

Advantages of ReadyAgarose™ Precast Gels Over 96-Well Handcast Gels for High-Throughput Analysis

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Introduction

Running PCR samples on agarose gels is a routine yet time-consuming procedure. It is common to hand cast a horizontal 96-sample gel for this purpose. A gel of such large size is difficult to cast, handle, and image, and requires a long run time. These issues can be resolved by using two of Bio-Rad's 2 x 32-well ReadyAgarose precast gels instead. ReadyAgarose gels are cast in their own UV-transparent running tray with fluorescent well numbers and a ruler for easy reference. Their gel trays have "autolock" tabs that snap into the dedicated wide mini ReadySub-Cell™ GT cell, so you can simply drop one of these precast gels into place and load your samples. ReadyAgarose gels fit most non-Bio-Rad wide mini subcells as well and thus do not require equipment from a specific manufacturer. ReadyAgarose gels are available in 0.8%, 1%, and 3% agarose in TBE or TAE buffer, with or without ethidium bromide, to fit your research requirements. In addition, two 2 x 32-well ReadyAgarose gels offer more wells than a 96-sample handcast gel, run in 30–45 min, and have 4.0 cm of resolving distance. These precast gels are designed to give the same results as traditional handcast gels in a fraction of the time.

Methods

Hand Casting a 96-Sample Gel

A gel was made by adding 8.7 g of Bio-Rad Certified™ low range ultra agarose (the same agarose used to manufacture

3% ReadyAgarose gels) to 290 ml of 1x TAE buffer and heating the mixture in a microwave oven to dissolve the agarose. The time required to melt the agarose was noted. The liquid was allowed to cool for 10 min, and 14.5 µl of 10 mg/ml ethidium bromide was added. The molten agarose was then poured into a 25 x 15 cm tray enclosed in a gel caster. Two combs, each with 51 wells, were placed in the tray. The agarose was then allowed to solidify and the time for this process was noted.

Preparing and Loading the Samples

Samples were prepared for electrophoresis by mixing 1.5 µl of a 500 bp PCR sample, 3.0 µl of 5x sample loading buffer containing Orange G, and 10.5 µl of 1x TAE buffer for each sample. The samples were loaded into the 102-well handcast gel and two 2 x 32-well, TAE 3% ReadyAgarose gels (with ethidium bromide) using a multichannel pipet. Several lanes on each gel were loaded with Bio-Rad's EZ Load™ 100 bp or 500 bp ruler as reference standards.

Running the Gels

The 102-well gel was run in a Sub-Cell® Model 96 cell at 100 V using the PowerPac™ 300 power supply. The two ReadyAgarose gels were run simultaneously in two wide Mini-Sub Cell GT systems, also at 100 V. TAE buffer (1x) was used to run all of the gels, and the volume of buffer used for each run was noted. In both cases the gels were run until the Orange G dye migrated to 3.5 cm from the well. The running times were noted for both the handcast and precast gels.

Imaging the Gels

Bio-Rad's Fluor-S® imager and Quantity One® 1-D image analysis software were used to image the gels. The handcast gel was imaged as one large gel. The ReadyAgarose gels were imaged as a composite.

Results

Comparative Differences

- To run the handcast gel, 2 L of 1x TAE was required, while only 1.2 L of the same buffer was needed to run the two ReadyAgarose gels
- It took 45 min to run the ReadyAgarose gels at 100 V, while it took 90 min to run the handcast gel at the same voltage
- When the gels were imaged, the background of the handcast gel was not uniform, suggesting that the ethidium bromide was not distributed uniformly, probably because of the high viscosity of a 3% agarose solution (Figure 1). However, no such unevenness was seen in the ReadyAgarose gels (Figure 2)
- With the numbered wells on the ReadyAgarose gel tray, documentation of lane samples was both easier and less prone to error. The ruler on the ReadyAgarose tray also facilitated easy and accurate measurement of band migration distances

Time and Cost Differences

The total time needed to prepare and run the handcast and precast gels is compared in Figure 3. A sample cost analysis for 3% gels is presented in Table 1.

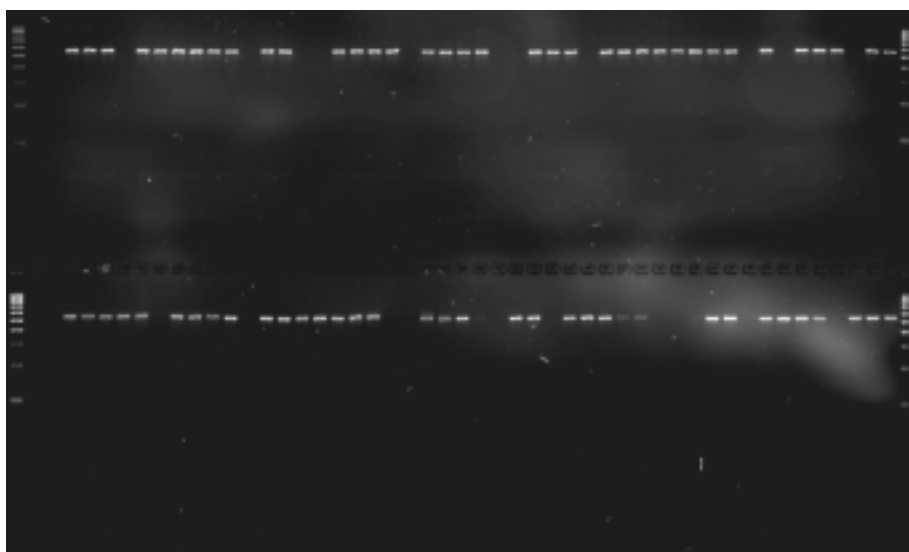


Fig. 1. A 102-well handcast 3% agarose gel with samples of 500 bp PCR products and 100 bp rulers in end lanes.

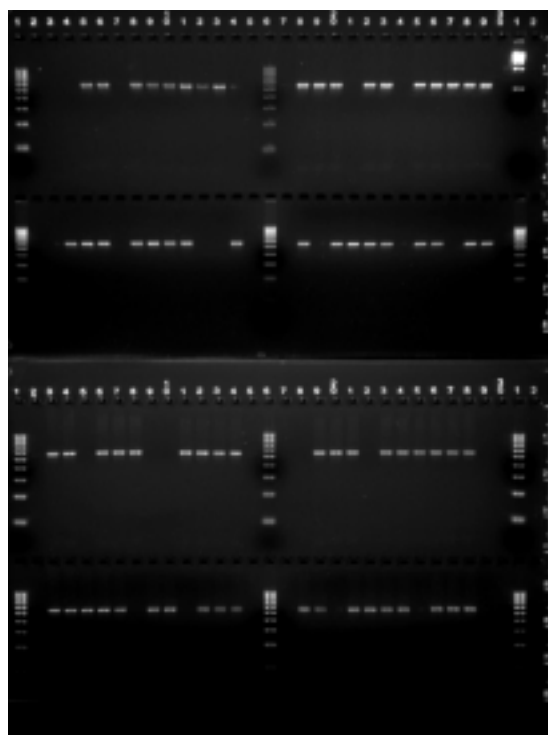


Fig. 2. Two 2 x 32-well, TAE 3% ReadyAgarose gels. Single-band samples are 500 bp PCR products. Far right lane of upper gel contains EZ Load 500 bp ruler; other standards are 100 bp rulers. Note the fluorescent well numbers and ruler to aid lane and band identification.

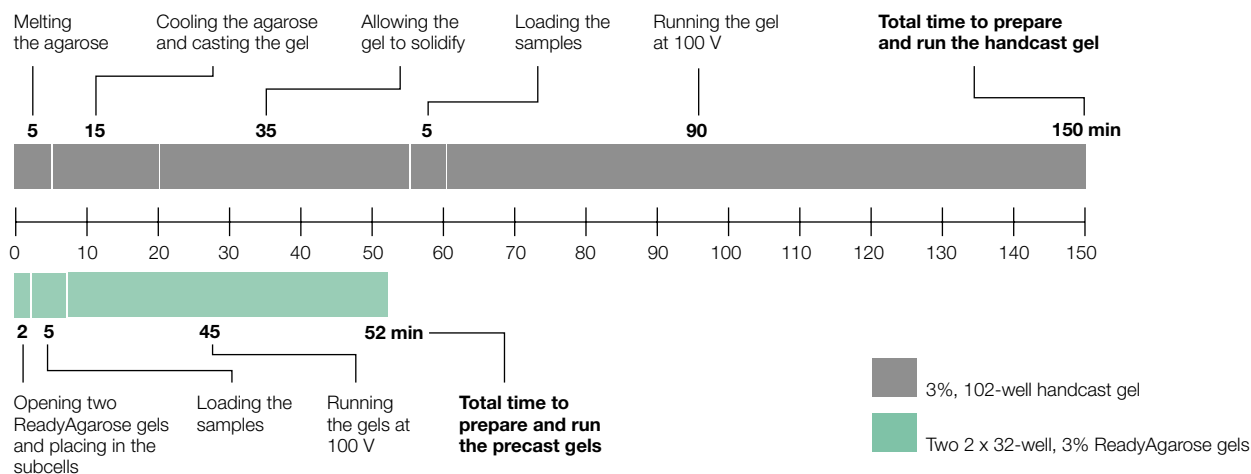


Fig. 3. Time comparison for gel preparation and electrophoresis of one handcast gel vs. two precast gels.

Table 1. Cost analysis (based on US list price).

Gels and Reagents	Unit Price, US \$	Cost of 102-well, 3% handcast gel (25 x 15 cm) US \$	Cost of 2 x 32-well, 3% ReadyAgarose precast gels (15 x 10 cm) US \$
2 x 32-well TAE			
3% ReadyAgarose gel with ethidium bromide	6.50 ea	N/A	13.00
8.7 g Certified™ low range ultra agarose	1.99/g	17.31	N/A
290 ml 1x TAE buffer (to prepare the gel)	54.00/L (50x)	0.32	N/A
14.5 µl ethidium bromide	30.00/10 ml	0.04	N/A
1x TAE buffer (to run the gels)	54.00/L (50x)	2.20	1.32
Total cost to cast and run gel(s)		\$19.87	\$14.32

Conclusions

In our tests, both handcast and precast gels gave equivalent band sharpness for PCR samples. However, the use of two 2 x 32-well ReadyAgarose precast gels instead of a large 96-sample (102-well) handcast gel saves preparation and running time. Two ReadyAgarose 2 x 32-well gels offer more lanes than a handcast gel designed specifically for 96 samples, thus allowing extra samples or standards to be included in a single run. Data from ReadyAgarose gels are easier to document since well numbers and a ruler are printed on the tray. These advantages of using two ReadyAgarose gels over one large handcast gel make them suitable and desirable for high-throughput laboratories where gel-to-gel consistency is important and relatively small differences in time can accumulate over the long run. Using ReadyAgarose precast gels may also be cost-effective, as this particular comparison demonstrates.

Ordering Information

TBE Gels	ReadyAgarose Mini Gels*			ReadyAgarose Wide Mini Gels*		
	8-Well (20 µl)	12-Well (10 µl)	2 x 8-Well (20 µl)	20-Well (20 µl)	32-Well** (15 µl)	2 x 32-Well** (15 µl)
0.8%	161-3001	161-3007	—	161-3025	161-3031	—
0.8% plus ethidium bromide	161-3002	161-3008	—	161-3026	161-3032	—
1%	161-3003	161-3009	—	161-3027	161-3033	161-3037
1% plus ethidium bromide	161-3004	161-3010	—	161-3028	161-3034	161-3038
3%	161-3005	161-3011	—	161-3029	161-3035	161-3039
3% plus ethidium bromide	161-3006	161-3012	—	161-3030	161-3036	161-3040
TAE Gels						
0.8%	161-3013	161-3019	—	161-3041	161-3047	—
0.8% plus ethidium bromide	161-3014	161-3020	—	161-3042	161-3048	—
1%	161-3015	161-3021	161-3057	161-3043	161-3049	161-3053
1% plus ethidium bromide	161-3016	161-3022	—	161-3044	161-3050	161-3054
3%	161-3017	161-3023	—	161-3045	161-3051	161-3055
3% plus ethidium bromide	161-3018	161-3024	—	161-3046	161-3052	161-3056

Gel shelf life at room temperature: All TAE gels with ethidium bromide, and TBE 3% plus ethidium bromide, 6 months; all other gels, 12 months.

*ReadyAgarose mini gels fit subcells at least 7.1 cm wide; wide mini gels fit subcells at least 15.6 cm wide.

**Compatible with multichannel pipets.

Catalog # Description

Molecular Rulers

170-8351 20 bp EZ Load Molecular Ruler, 20–1,000 bp range, 100 applications
 170-8352 100 bp EZ Load Molecular Ruler, 100–1,000 bp range, 100 applications
 170-8200 AmpliSize® Molecular Ruler, 50–2,000 bp range, 50 applications
 170-8353 100 bp PCR EZ Load Molecular Ruler, 100–3,000 bp range, 100 applications
 170-8354 500 bp EZ Load Molecular Ruler, 500–8,000 bp range, 100 applications
 170-8355 1 kb EZ Load Molecular Ruler, 1–15 kb range, 100 applications
 170-8205 2.5 kb Molecular Ruler, 2.5–35 kb range, 100 applications
 170-8356 EZ Load Precision Molecular Mass Ruler, 100–1,000 bp range, 10–100 ng, 100 applications

Premixed Buffers

161-0767 5x Nucleic Acid Sample Buffer, 10 ml
 161-0733 10x TBE, 1 L
 161-0770 10x TBE, 5 L cube
 161-0743 50x TAE, 1 L
 161-0773 50x TAE, 5 L cube
 161-0774 20x SSC, 1 L
 161-0775 20x SSC, 5 L cube

Submerged Horizontal Electrophoresis Cells

170-4487 Mini ReadySub-Cell™ GT Cell, includes subcell unit only
 170-4489 Wide Mini ReadySub-Cell GT Cell, includes subcell unit only

Ethidium Bromide Solutions and Tablets

161-0433 Ethidium Bromide Solution, 10 ml, 10 mg/ml
 161-0430 Ethidium Bromide Tablets, 10 x 10 mg

Power Supplies

165-5050 PowerPac 300 Power Supply, 100/120 V, with 300 V, 400 mA output capacity
 165-5051 PowerPac 300 Power Supply, 220/240 V, with 300 V, 400 mA output capacity
 165-5052 PowerPac 200 Power Supply, 100/120 V, with 200 V, 2.0 A output capacity
 165-5053 PowerPac 200 Power Supply, 220/240 V, with 200 V, 2.0 A output capacity

The polymerase chain reaction (PCR) process is covered by patents owned by Hoffman-LaRoche. Use of the PCR process requires a license.

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