Automated Plasmid Purifications using the MagBinder® Fit^{24}: Mini, Midi, and More

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MagBinder® Fit^{24} Nucleic Acid Purification System

Figure 1. MagBinder® Fit^{24} Nucleic Acid Purification System

The MagBinder® Fit^{24} is a new benchtop nucleic acid purification system designed to meet the needs of low-to-moderate throughput users by providing semi-automated purification of 1 to 24 small or large volume sample inputs. This automation platform can support various DNA and RNA workflows, sample versatility, and flexible input volumes to achieve consistent, high-quality results.

Plasmid Purification Made Easy

The MagBinder® Fit^{24}, in conjunction with magnetic bead-based purification kits, like the Mag-Bind® Endo-free Plasmid Mini Kit (M1261) and Mag-Bind® Endo-free Plasmid Midi Kit (M1272), offers a scalable, one-stop solution for plasmid purification encompassing mini to midi preps. Up to 50 mL bacterial cultures grown in Luria-Bertani (LB) medium or up to 10 mL bacterial cultures grown in Terrific Broth (TB) can be processed using this workflow. The reagents from the purification kits are user-filled into 5 mL (PB07-5-200) or 10 mL (PB05-10-200) reagent cartridges. The cartridge layout for Mag-Bind® Endo-free Plasmid Mini and Midi kits is shown in **Figure 2**. The Mag-Bind® Endo-free Plasmid Mini and Midi Kits follow a modified alkaline lysis method forming a bacterial lysate. The lysate is then cleared using either lysate clearance beads (Mag-Bind® Particles LC) or centrifugation. Lysate Clearance Syringe can also be used in the case of a midiprep. The upfront alkaline lysis and lysate clearance are carried out offline. The lysate is transferred to Well 1 of the reagent cartridge and subsequent bind-wash-elute steps are performed on the MagBinder® Fit^{24}. Eluted plasmid DNA is high-quality, endotoxin-free with endotoxin levels < 0.1 EU/µg. The information about the plasmid purification kits and their specifications are shown in **Table 1**.

Plasmid Purification: Minipreps

Plasmid Purification from 1.5 mL LB, TB, and 2xYT
E. coli strain DH5α harboring high copy number plasmid pGL4.51 was grown overnight in 2xYT or TB media. DH5α harboring pGEM was grown overnight in LB medium concurrently. Plasmid DNA was purified using Mag-Bind® Endo-free Mini Kit on the MagBinder® Fit^{24} and quantified by absorbance using Thermo Scientific's NanoDrop™ 2000c system. Run time was ~65 minutes. Yield and purity of plasmid DNA is shown in **Figure 3**. The results corroborate the versatility of the extraction workflow that is capable of handling diverse sample input conditions ranging from plasmid types, plasmid sizes, culture medium, and input culture volumes.

Table 1. Plasmid Kits and Specifications for automating on MagBinder® Fit^{24} Platform

Kit	Input Culture Volume	Reagent Catridge To Use	Lysate Clearance Methodology	Elution Volume	*Processing Time on MagBinder® Fit^{24}
Mag-Bind® Endo-free Plasmid Mini Kit	Up to 1.5 mL LB or TB	MB Fit24™ Reagent Cartridge, 5 mL	Centrifugation or Mag-Bind® Particles LC	50-100 µL	~65 min
Mag-Bind® Endo-free Plasmid Midi Kit	Up to 5 mL LB	MB Fit24™ Reagent Cartridge, 10 mL	Centrifugation or Mag-Bind® Particles LC or Lysate Clearance Syringe	100 µL	~70 min
Mag-Bind® Endo-free Plasmid Midi Kit	Up to 50 mL LB or 10 mL TB	MB Fit24™ Reagent Cartridge, 10 mL		500-1000 µL (250 µL for < 25 mL culture volume input)	~70 min

***Processing time when starting from a bacterial pellet, including the offline steps**

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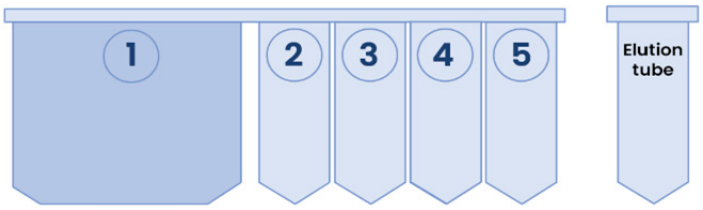
5 mL Reagent Cartridge Layout for Minipreps
(up to 1.5 mL bacterial culture)

10 mL Reagent Cartridge Layout for Midipreps
(up to 50 mL bacterial culture)



Position	Contents	Mag-Bind® Endo-free Plasmid Mini Kit (M1261) - up to 1.5 mL culture input	Mag-Bind® Endo-free Plasmid Midi Kit (M1272) - up to 5 mL culture input	Mag-Bind® Endo-free Plasmid Midi Kit (M1272) - up to 50 mL culture input
1	IRD Buffer + Mag-Bind® Particles RQ	500 µL IRD Buffer + 20 µL Mag-Bind® Particles RQ	2.5 mL IRD Buffer + 50 µL Mag-Bind® Particles RQ	5.5 mL IRD Buffer + 200 µL Mag-Bind® Particles RQ
2	IRD Buffer	500 µL	1 mL	1.25 mL
3	VHB Buffer	700 µL	1 mL	1.25 mL
4	VHB Buffer	700 µL	1 mL	1.25 mL
5	70% Ethanol	700 µL	1 mL	1.25 mL
6	Empty	Empty	Empty	Empty
7	Empty	Empty	Empty	Empty
Elution Tube	Endo-free Water	50 - 100 µL	100 µL	500 - 1000 µL

Figure 2. MagBinder® Fit[24] reagent cartridge positions, contents, volume used in Mag-Bind® Endo-free Plasmid Mini and Midi Kits.

Versatility Across Various Culture Media and Plasmid Types

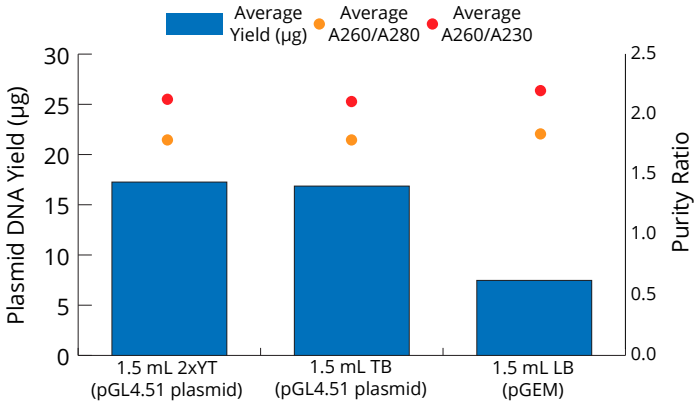


Figure 3. Mag-Bind® Endo-free Plasmid Mini Kit on MagBinder® Fit[24] is capable of handling diverse sample input conditions ranging from plasmid types, plasmid sizes, culture medium, and input culture volumes. The plasmid yields may vary based on these conditions.

Comparison Data vs Company P Using 1.5 mL Input Culture Volume
pGEM plasmid was purified from 1.5 mL DH5α cultures (n=3) using Mag-Bind® Endo-free Plasmid Mini Kit automated on MagBinder® Fit[24] and a comparable magnetic bead-based kit from Company P, following manufacturer's recommended protocol. Purified Plasmid DNA was quantified using Thermo Scientific's NanoDrop™ 2000c system. Plasmid preparation was not only endotoxin-free (<0.1 EU/µg) for Omega Bio-tek's kit, but the yield was also significantly higher (~1.8X) compared to Company P's kit (**Table 2**).

Table 2. ~1.8X higher plasmid yield vs Company P

Manufacturer	Yield (µg)	A260/ A280	A260/ A230	Endotoxin levels (EU/µg)
Omega Bio-tek	7.0	1.84	1.98	0.02 (endotoxin-free)
Company P	3.9	1.84	1.98	0.14

Downstream Application of Transfection
Plasmid pGL4.51 which encodes the luciferase gene was prepared using both Omega Bo-tek's Mag-Bind® Endo-free Plasmid Mini Kit automated on MagBinder® Fit[24] and Company P's Kit, following the manufacturer's recommended protocol. 200 ng of purified plasmid using both methodologies was transfected into 10,000 cells using 2 µL Lipofectamine Transfection Reagent. Plasmid purified using Omega Bio-tek's Kit resulted in higher bioluminescent signal, indicating higher transfection efficiency, compared to plasmid purified using Company P's kit (**Figure 4**). The higher transfection efficiency achieved using Omega Bio-tek's workflow is likely because the plasmid purification was endotoxin-free using Omega Bio-tek's kit.

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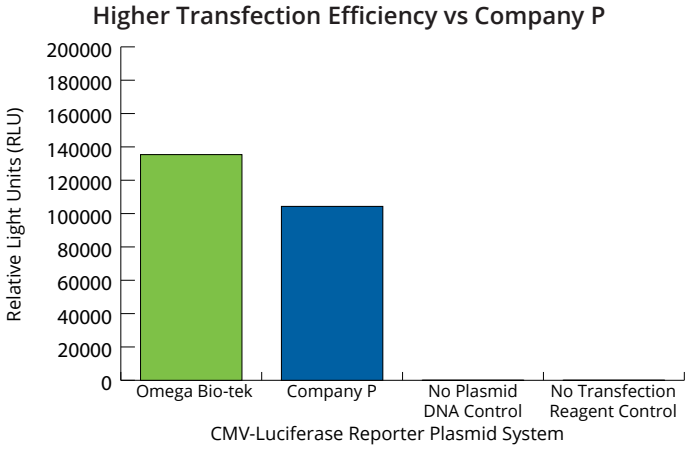

Figure 4. Higher transfection efficiency using Omega Bio-tek's workflow compared to Company P, owing to the plasmid purification being endotoxin-free using Omega Bio-tek's kit.

Plasmid Purification: Midipreps

Comparison data vs Company T using 5 mL input culture volume
pGEM and pGL4.51 plasmid were purified from 5 mL DH5α cultures (n=3) using Mag-Bind® Endo-free Plasmid Midi Kit on the MagBinder® Fit[24] and Company T's Kit, following manufacturer's recommended centrifugation protocol. Purified Plasmid DNA was quantified using Thermo Scientific's NanoDrop™ 2000c system. Plasmid preparation was not only endotoxin-free (< 0.1 EU/µg) for Omega Bio-tek's kit, but the yield was also significantly higher compared to Company T's kit (**Table 3**).

Table 3. Significantly higher plasmid yields compared to Company T using 5 mL input culture volume.

Plasmid	Manufacturer	Yield (µg)	A260/A280	A260/A230	Endotoxin levels (EU/µg)
pGEM (~3.3 kb)	Company T	9.0	1.9	2.4	0.651
	Omega Bio-tek	31.7	1.8	2.1	<0.01 (endo-free)
pGL4.51 (high-copy) (~6.4 kb)	Company T	10.2	1.9	2.3	Not Available
	Omega Bio-tek	87.5	1.8	2.2	Not Available

Downstream Application of Transfection
200 ng of plasmid pGL4.51, prepared as described above, was transfected into 10,000 cells using 2 µL Lipofectamine Transfection Reagent. Plasmid purified using Omega Bio-tek's Kit resulted in higher bioluminescent signal, indicating higher transfection efficiency compared to plasmid purified using Company T's kit (**Figure 5**).

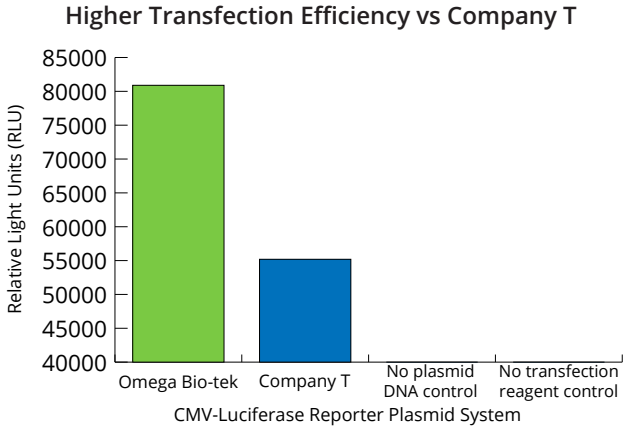

Figure 5. Higher transfection efficiency using Omega Bio-tek's workflow compared to Company T owing to the plasmid purification being endotoxin-free using Omega Bio-tek's kit.

Plasmid Purification from 50 mL LB and 10 mL TB
E. coli strain DH5α harboring pGEM was grown overnight in 25 mL, 50 mL LB, or 10 mL TB media, following three different lysate clearance methodologies (centrifugation, Mag-Bind® Particles LC, and lysate clearance syringe). Plasmid DNA was purified using Mag-Bind® Endo-free Plasmid Midi Kit on MagBinder® Fit[24] and quantified by absorbance using Thermo Scientific's NanoDrop™ 2000c system. Run time was roughly ~70 min. Yield and purity of plasmid DNA is shown below in **Figure 6**. Yields were comparable irrespective of the lysate clearance methodology used. Plasmid yields roughly doubled going from culture volumes 25 mL to 50 mL and had excellent purity ratios with A260/A280 ~1.8 and A260/A230 ~2.2. The purified plasmids, irrespective of the culture volume or culture medium, were endotoxin-free with endotoxin levels < 0.1 EU/µg.

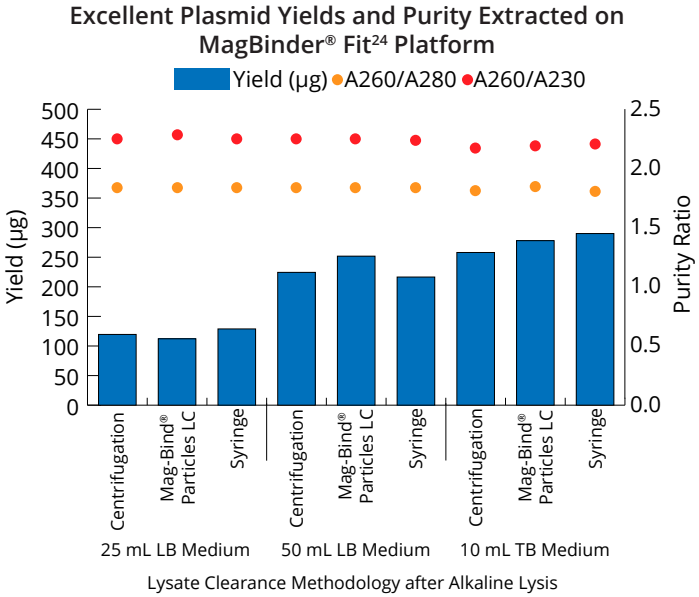

Figure 6. pGEM plasmid was purified from 25 mL and 50 mL LB as well as 10 mL TB media (n=3) using Mag-Bind® Endo-free Plasmid Midi Kit automated on the MagBinder® Fit[24] Platform following three different lysate clearance methodologies. Yields and quality were comparable, irrespective of the lysate clearance methodology used.

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Conclusions

MagBinder® Fit^{24} Nucleic Acid Purification System, in conjunction with Omega Bio-tek's magnetic bead-based plasmid kits, offers an attractive, all-encompassing solution covering the plasmid purification spectrum from mini to midi preps. Purified plasmid is endotoxin-free that can be used in myriad applications, ranging from sensitive cell transfections to routine screening.

Product Information

Product	Description
MagBinder® Fit^{24}	B1-001-24
MB Fit24™ Reagent Cartridge, 5 mL	PB07-5-200, 200 Cartridges
MB Fit24™ Reagent cartridge, 10 mL	PB05-10-200, 200 Cartridges
MB Fit24™ Tip Comb	PB12-0-72
Elution Tube (2 mL)	PB01-2-500
Mag-Bind® Endo-free Plasmid Mini Kit	M1261-00 (1 x 96) M1261-01 (4 x 96)
Mag-Bind® Endo-free Plasmid Midi Kit	M1272-00 (1 x 96) M1272-01 (4 x 96)
Mag-Bind® Particles LC	MBPLC-50
Lysate Clearance Syringe	FLMidi-03

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