



Liquid Biopsy Research - "We are where you start"



SCIENCE IS OUR BUSINESS

HighPrep™ Plasmid DNA Plus Kit

Manual Revision v2.04

Catalog Nos. HPPL-DP5, HPPL-DP96, HPPL-DP96X4, HPPL-DP96X20, HPPL-DP96x40

- DNA isolation of low/high copy plasmid DNA, BACs, PACs, Cosmid and Fosmids
- Magnetic beads based chemistry

PROTOCOL

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Product Description

The HighPrep™ Plasmid DNA Plus Kit utilizes magnetic beads with alkaline-SDS lysis of bacterial cells to deliver high-quality plasmid DNA in a high-throughput format. By using a 96-well format, up to 96 samples can be simultaneously processed in less than 60 minutes. By utilizing magnetic beads chemistry, the system is geared for automation as the protocol requires no vacuum filtration. When used with high copy plasmids, the HighPrep™ Plasmid DNA kit yields ~10-15 µg. Yields vary slightly depending on the cell line, vector type, and the size of the construct. Purified plasmid DNA can be used in the following applications:

- Fluorescent DNA sequencing
- PCR amplification
- Transformation
- Restriction enzyme digestion

Process

Pelleted *E.coli* cells are resuspended in SOL1 and lysed with SOL2. The Neutralization Buffer causes the *E.coli* chromosomal DNA and cellular contaminants to coagulate and form a flocculent material. The clear lysate containing plasmid DNA is transferred out from under the flocculent material to a new processing plate. HighPrep™ MAG-S1 magnetic particles are added to bind to Plasmid DNA. Beads are washed twice and eluted for downstream application. A magnetic separation plate is used for separating the beads from the solution.

Kit Contents and Storage

HighPrep™ Plasmid DNA Plus Kit Catalog No.	HPPL-DP5	HPPL-DP96	HPPL-DP96X4	HPPL-DP96X20	HPPL-DP96X40	STORAGE
Number of Preps	5	96	384	1920	3840	15-25°C
SOL1 ¹	0.6 mL	15 mL	60 mL	300 mL	625 mL	15-25°C
SOL2	0.6 mL	15 mL	60 mL	300 mL	625 mL	15-25°C
Neutralization Buffer	0.6 mL	15 mL	60 mL	300 mL	625 mL	15-25°C
MB Elution Buffer	1 mL	10 mL	50 mL	250 mL	625 mL	15-25°C
RNase A	0.0033 mL	100 µL	400 µL	2 mL	4.2 mL	2-8°C
MAG-S1 Particles	0.055 mL	1.1 mL	4.2 mL	21 mL	46 mL	2-8°C

¹ RNase A must be added prior to use. See Preparation of Reagents and Storage Conditions on page 2.

Stability

All components are stable for 14 months when stored accordingly.

Preparation of Reagents

Prepare the following components for each kit before use:

Catalog No.	Component	Add RNase A	Storage
HPPL-DP5	SOL1	RNase A already added (included with kit)	2-8°C
Components are stable for 14 months when stored accordingly			

Catalog No.	Component	Add RNase A	Storage
HPPL-DP96	SOL1	Add 100 µL RNase A (included with kit)	15-25°C
Components are stable for 14 months when stored accordingly			

Catalog No.	Component	Add RNase A	Storage
HPPL-DP96X4	SOL1	Add 400 µL RNase A (included with kit)	15-25°C
Components are stable for 14 months when stored accordingly			

Catalog No.	Component	Add RNase A	Storage
HPPL-DP96X20	SOL1	Add 2 mL RNase A (included with kit)	15-25°C
Components are stable for 14 months when stored accordingly			

Catalog No.	Component	Add RNase A	Storage
HPPL-DP96X40	SOL1	Add 4.2 mL RNase A (included with kit)	15-25°C
Components are stable for 14 months when stored accordingly			

HighPrep™ Plasmid DNA Plus Kit - 96 format protocol

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). MSDS can be downloaded from the "Product Resource" tab when viewing the product kit.

Equipment and Reagents to Be Supplied by User

- 96 well ring magnet separation device
- Source Plate: 96-well 2.2 mL deep well culture plate
- Destination Plate: 300 µL round bottom microtiter plate
- Gas permeable seals
- Incubator set at 60°C
- 96-100% Ethanol
- 70% Ethanol

Things to do before starting

- Ensure SOL1 is prepared according to the instructions on page 2.
- Preheat MB Elution Buffer to 55°C.
- Prechill Neutralization Buffer to 4°C.

Protocol

- 1. Grow 1.0-1.5 ml E.coli LB cultures in a 2 mL 96-well culture plate at 37°C with agitation for 16-20 hours.**
- 2. Seal plate with sealing film and pellet bacterial cultures by centrifuging the culture plate at 3,000 x g for 10 minutes.**
- 3. After centrifugation, remove the sealing film and and discard the supernatant. Blot the inverted culture plate on a paper towel to remove excess media.**
- 4. Add 100 µL of SOL1 and thoroughly resuspend the cell pellets by pipetting up and down or by vortexing.**
Pipette mix 20 times. If vortexing, vortex for 2-3 minutes at high setting. Vortexing will take 2-3 minutes on a high setting. The mixture should appear homogenous and should not have any cell clumps.
- 5. Add 100 uL of SOL2 and gently mix by shaking the plate for 1 min and allowing the plate to incubate for 5 minutes.**
Shake on an orbital shaker at 300-600 RPM. Alternatively, gently pipette mix 2 times then allow the samples to sit for 5 minutes for complete lysis. Avoid vigorous pipette mixing as large plasmids can be easily sheared and this may affect sample integrity. Do not allow samples to lyse for longer than 10 minutes.

 Store SOL2 tightly capped when not in use.

- 6. Add 100µL of chilled (4°C) Neutralization Buffer, gently mix by shaking, and allow samples to neutralize for 10 min. White flocculent precipitates will form.**

Shake the samples for 10 minutes at 300-600 RPM to complete the neutralization. Alternatively, pipette mix very gently near the bottom of the plate, avoiding the flocculent material at the top of the well.

- 7. Transfer 110 µL of the clear lysate near the bottom of the well to a 300 µL round bottom microtiter plate.**

⚠ The transfer of the clear lysate is a critical step of the process. The supernatant should be free of flocculent material for optimal results.

Alternatively, centrifugation of the lysate to compact the flocculent is recommended for ease of transfer and optimal results.

- Centrifuge the samples at 5000 x *g* for 20 minutes to pellet the flocculent material.
- Slowly aspirate and transfer 110 µL of the clear lysate from the top of the well to a new clean round bottom plate. Avoid touching and transferring any of the pelleted flocculent material.

- 8. Add 10 µL of MAG-S1 particles and 80µL of 100% Ethanol to each well. Pipette mix 20 times.**

⚠ Shake well to resuspend the MAG-S1 particles before use.

- 9. Incubate the plate for 5 min at room temperature.**

⚠ For low copy number plasmid, longer incubation may increase the yield.

- 10. Place the plate on the magnetic separation device and allow the beads to magnetize for 5 min or until the solution is clear.**

⚠ The supernatant may have slight yellow-brown tint but should not be cloudy.

- 11. With the plate on the magnet, remove and discard the supernatant by pipetting.**

⚠ Do not disturb the attracted beads while aspirating the supernatant.

- 12. Remove the plate off the magnet. Add 250 µL of 70% ethanol (freshly prepared) to each well of the plate and pipette mix 10 times**

- 13. Place the plate back on the magnet and allow beads to magnetize for 5 min or until the solution is clear.**

- 14. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.**

⚠ Do not disturb the attracted beads while aspirating the supernatant.

- 15. Repeat steps 12-14 for a second, and third wash.**

- 16. With the plate on the magnet, dry the plate at room temperature for 7 minutes or until all residual liquid has evaporated.**

- 17. Remove the plate off the magnet, add 50 µL of MB Elution Buffer, and pipette mix 10 times.**

⚠ Prewarming the MB Elution Buffer at 55°C can increase the yield.

- 18. Incubate the plate at room temperature for 1-2 min.**
- 19. Place the sample plate back on the magnet and wait for 5 min or until the magnetic beads clear from the solution.**
- 20. Transfer the eluate (cleared supernatant containing the DNA) to a new microplate for storage. Store DNA at -20°C.**

Troubleshooting guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via:

Phone: 1-855-262-4246 (in US), outside US, 1-301-302-0144

Email: support@magbiogenomics.com

Problem	Possible Cause	Action
Low DNA yield	Poor cell lysis	Do not use more than 1 mL with high copy plasmids.
		Cells may not be dispersed adequately prior to addition of SOL1. Vortex cell suspension to completely disperse.
		Increase incubation time with SOL2 to obtain a clear lysate.
		SOL2 needs to be stored tightly. If not, it may need to be replaced.
	Bacterial culture overgrown or not fresh.	Do not incubate cultures for more than 16 hr at 37°C.
	Low copy-number plasmid used	Such plasmids may yield as little as 0.1 µg DNA from a 1 ml overnight culture.
High-molecular weight DNA contamination	Over mixing of cell lysate upon addition of SOL2	Do not vortex or aggressively mix after adding SOL2.
Optical densities do not agree with DNA yield on agarose gel	Trace contaminants eluted from column increase A260	Make sure to wash the MAG-S1 particles as instructed. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantification.
RNA visible on agarose gel	RNase A not added to SOL1	Prepare SOL1 as instructed on Preparation of Reagents section.
DNA floats out of well while loading agarose gel	Ethanol not completely removed before elution	Increase air dry time before elution step.

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use. All biological samples are considered potentially infectious. When working with the samples and chemicals always wear a suitable lab coat, disposable gloves, and protective goggles. For more information please consult the appropriate Material Safety Data Sheets (MSDSs).



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