MAGBIO

Liquid Biopsy Research - “We are where you start”

HighPrep™ Plasmid DNA Kit

Catalog Nos. HPPL-D10, HPPL-D96, HPPL-D96X4, HPPL-D96X40
Manual Revision v2.04

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

PROTOCOL

Contents

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

Information in this document is subject to change without notice.

Product Description

The HighPrep™ Plasmid DNA Kit utilized 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 vaccuum filtration. When use with high copy plasmids, the HighPrep Plasmid DNA kit yields ~10-15 µg. Yields vary slightly depending on the cell line, vector type, and 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 solution. The Neutralization Buffer causes the E.*coli* chromosomal DNA and cellular contaminates 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. Beads are washed twice and finally eluted for downstream application. A magnetic separation plate is used for separating the beads from solution.

Kit Contents and Storage

HighPrep™ Plasmid DNA Kit Catalog No.	**HPPL-D10**	**HPPL-D96**	**HPPL-D96X4**	**HPPL-D96X40**	**STORAGE**
Number of Preps	10	96	384	3,840	15-25°C
SOL1[1]	1.2 mL	15 mL	60 mL	625 mL	15-25°C
SOL2	1.2 mL	15 mL	60 mL	625 mL	15-25°C
Neutralization Buffer	1.2 mL	15 mL	60 mL	625 mL	15-25°C
MB Elution Buffer	1.2 mL	10 mL	50 mL	625 mL	15-25°C
RNase A	10 µL	100 µL	400 µL	4.2 mL	2-8°C
MAG-S1 Particles	114 µL	1.1 mL	4.2 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 12 months when stored accordingly.

Preparation of Reagents

Prepare the following components for each kit before use:

Catalog No.	Component	Add RNase A	Add 96-100 % Ethanol	Storage
HPPL-D10	SOL1	Add 10 µL RNase A (included with kit)	- - -	2-8°C
Components are stable for 1 year when stored accordingly				

Catalog No.	Component	Add RNase A	Add 96-100 % Ethanol	Storage
HPPL-D96	SOL1	Add 100 µL RNase A (included with kit)	- - -	2-8°C
Components are stable for 1 year when stored accordingly				

Catalog No.	Component	Add RNase A	Add 96-100 % Ethanol	Storage
HPPL-D96X4	SOL1	Add 400 µL RNase A (included with kit)	- - -	2-8°C
Components are stable for 1 year when stored accordingly				

Catalog No.	Component	Add RNase A	Add 96-100 % Ethanol	Storage
HPPL-D96X20	SOL1	Add 2 mL RNase A (included with kit)	- - -	2-8°C
Components are stable for 1 year when stored accordingly				

HighPrep™ Plasmid DNA 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 capable of 60°C
- ☐ Isopropanol
  Ethanol (96-100%)

Things to do before starting

- ☐ Ensure SOL1 is prepared accordingly 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 with for 16-20 hours.**

2. **Seal plate with sealing film and pellet bacterial cultures by centrifuging the culture plates 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 SOL1 and thoroughly resuspend the cell pellets by pipetting up or 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 appears homogenous and should not have any cell clumps.

5. **Add 100 uL 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 lower purity. 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 and 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 MAG-S1 particles and 80µL of 100% Isopropanol to each well. Pipette mix 20 times.**
   ⚠ Shake well to resuspend the MAG-S1 particles before use.

9. **Incubate plate for 5 min at room temperature.**
   For low copy number plasmid, longer incubation may increase yield.

10. **Place the plate on the magnetic separation device and allow 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 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 for 7 minutes or until all residual liquid has evaporated.**

17. **Remove the plate off the magnet and add 50 µL MB Elution Buffer and pipette mix for 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 5 min or until the magnetic beads clear from 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 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.

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