MAGBIO

"WE MAKE NGS BETTER"

HighPrep Plasmid DNA Plus Kit - DX

Isolation of Plasmid DNA

Catalog Nos. HPPL-DP5E, HPPL-DP96E, HPPL-DP96x4E, HPPL-DP96x20E, HPPL-DP96x40E
Manual Revision 0
WI-72-63

- Isolation of low/high copy plasmid DNA, BACs, PACs, Cosmids, and Fosmids
- Magnetic bead-based chemistry

Instructions For Use

Contents

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CE IVD MHRA

For *in vitro* diagnostic procedures.

Information in this document is subject to change without notice.

Product Description

The HighPrep Plasmid DNA Plus Kit - DX 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 vaccuum filtration. When used with high copy plasmids, the HighPrep Plasmid DNA Plus Kit - DX 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:

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

This product is intended to be used by qualified and trained laboratory professionals only.

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. MAG-S1 Particles are added to bind to plasmid DNA. Beads are washed 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 - DX Catalog No.*	HPPL-DP5E	HPPL-DP96E	HPPL-DP96x4E	HPPL-DP96x20E	HPPL-DP96x40E	Storage
Number of Preps	5	96	384	1920	3840	
SOL1[1]	600 µL (Includes RNase A)	15 mL	60 mL	300 mL	625 mL	15-25°C
SOL2	600 µL	15 mL	60 mL	300 mL	625 mL	15-25°C
Neutralization Buffer	600 µL	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[2]	3.3 µL (Included in SOL1)	100 µL	400 µL	2 mL	4.2 mL	2-8°C
MAG-S1 Particles[3]	55 µL	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 section.
*Once opened, reagents are usable until the expiration date on the product label. Be sure to close the lid firmly before storing reagents for later use.

Shipping and Storage

- [2] RNase A ships at room temperature. Store at 2-8°C.
- [3] MAG-S1 Particles ship at room temperature. Store at 2-8°C.
- Once combined, the mixture of RNase A and SOL1 is stored at 2-8°C.

Safety Information

Any consumables, including plates, tubes, etc., used to process samples with infectious or microbial hazards should be disposed of in an appropriate biohazard waste bin. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). The SDS can be downloaded from the "Product Documents" tab when viewing this product at www.magbiogenomics.com.

Preparation of Reagents

Prepare the following components for each kit before use:

Catalog No.	Component	Add RNase A*	Storage
HPPL-DP5E	SOL1	Add 3.3 µL RNase A (already added to SOL1)	2-8°C

Catalog No.	Component	Add RNase A*	Storage
HPPL-DP96E	SOL1	Add 100 µL RNase A (included with kit)	2-8°C

Catalog No.	Component	Add RNase A*	Storage
HPPL-DP96x4E	SOL1	Add 400 µL RNase A (included with kit)	2-8°C

Catalog No.	Component	Add RNase A*	Storage
HPPL-DP96x20E	SOL1	Add 2 mL RNase A (included with kit)	2-8°C

Catalog No.	Component	Add RNase A*	Storage
HPPL-DP96x40E	SOL1	Add 4.2 mL RNase A (included with kit)	2-8°C

**Ensure bottle/tube lid Is closed tightly when preparing and storing reagents.*

Additional Information

Bacterial Strain Selection

It is recommended that an EndA negative strain of E. coli be used for routine plasmid isolation. Examples of such strains include DH5α, DH1, and C600. These host strains yield high-quality Plasmid DNA with this protocol.

Host strains derivatives from HB101 such as TG1 and the JM100 series release large amounts of carbohydrates during lysis, which may inhibit enzyme activities if not completely removed. Some strains may also lower DNA quality due to having high levels of endonuclease activity, and therefore are not recommended (i.e. JM101, JM110, HB101).

Bacterial Culture Growth

Bacterial cultures for plasmid preparations should always be grown from a single colony picked from a freshly streaked plate. Subculturing directly from glycerol stock or liquid cultures may lead to uneven yields or plasmid loss. Optimal results are obtained by using one single isolated colony from a freshly transformed or freshly streaked plate to inoculate an appropriate volume of starter culture containing the appropriate antibiotic, and then incubated for 12-16 hours at 37°C with vigorous shaking (~250 rpm, shaking incubator). Aeration is very important. The culture volume should not exceed 1/4 the volume of the container.

This product is specially designed for use with cultures grown in Luria Bertani (LB) medium. Richer broths such as TB (Terrific Broth) or 2 x YT led to high cell densities that can overload the purification system, and are not recommended. If rich media must be used, growth times should be optimized, and the recommended culture volumes may be reduced to match the capacity of the MAG-S1 Particles or use more beads.

For optimal plasmid yields, the starting culture volume should be based on culture cell density. A bacterial density between 2.0 and 3.0 at OD600 is recommended. When using nutrient-rich media, care should be taken to ensure that the cell density does not exceed an OD600 of 3.0. Using a high-density culture outside of the recommended OD range may overload the purification system.

Harvest the Bacteria

Harvest cells by centrifugation at 10,000 x g for 10 minutes. Discard the supernatant. The bacterial pellet can be used immediately or stored at -20°C.

Protocol: Cultured Cells 1-1.5 mL sample volume (96 well format)

Equipment and Reagents to Be Supplied by the User

- ☐ 96 well ring magnet separation device
- ☐ Source Plate: 96-well 2.0 mL deep well culture plate
- ☐ Destination Plate: 500 µL round bottom microtiter plate
- ☐ Gas permeable sealing film
- ☐ Incubator capable of 55°C
- ☐ Ethanol (100%)
- ☐ Ethanol (70%)
- ☐ Centrifuge

Things to do Before Starting

- ☐ Ensure SOL1 is prepared according to the instructions on page 2.
- ☐ Preset incubator to 55°C.
- ☐ Prechill Neutralization Buffer to 4°C.

Protocol

⚠ Bring the **MAG-S1 Particles** to room temperature for at least 30 minutes before use.

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 12-16 hours.

2. Seal the plate with sealing film and pellet the 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/RNase A** and thoroughly resuspend the cell pellets by pipetting up and down 20 times or by vortexing. If vortexing, vortex for 2-3 minutes at high setting. Incubate at room temperature for 5 minutes. The mixture should appear homogenous and should not have any cell clumps.

   ⚠ ***RNase A** must be added to **SOL1** before use.*

5. Add 100 uL of **SOL2** and gently mix by shaking the plate on an orbital shaker at 300-600 RPM for 1 minute and allow the plate to incubate for 5 minutes. Alternatively, gently pipette mix 2 times, then allow the samples to sit for 5 minutes for a 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 5 minutes.

   ⚠ *Store **SOL2** tightly capped when not in use.*

6. Add 100 µL of chilled (4°C) **Neutralization Buffer** and gently mix by shaking for 10 minutes on an orbital shaker at 300-600 RPM to complete the neutralization. White flocculent precipitates will form. Alternatively, pipette mix very gently near the bottom of the plate, avoiding the flocculent material at the top of the well. Incubate at room temperature for 10 minutes to complete the neutralization.

7. Centrifuge the samples at 5000 x g for 10 minutes to pellet the flocculent material.

8. Slowly aspirate and transfer 200 µL of the clear lysate from the top of the well to a new clean 500 µL round bottom microtiter plate. Avoid touching and transferring any of the pelleted flocculent material.

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

9. Add 10 µL of **MAG-S1 Particles** and 200 µL of 100% Ethanol to each well. Pipette mix 20 times.

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

10. Incubate the plate for 5 minutes at room temperature with occasional mixing or shake the sample on an orbital shaker at 200-600 RPM during incubation.

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

11. Place the plate on the magnetic separation device and allow the beads to magnetize for 2 minutes or until the solution is clear.

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

12. With the plate on the magnet, remove and discard the supernatant by pipetting.

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

13. Remove the plate from the magnet. Add 400 µL of 70% Ethanol (freshly prepared) to each well of the plate and pipette mix 20 times.

14. Place the plate back on the magnetic separation device and allow the beads to magnetize for 2 minutes or until the solution is clear.

15. 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.*

16. Repeat steps 13-15 for a second, and third wash.

17. With the plate on the magnet, dry the plate at room temperature for 5 minutes or until all residual liquid has evaporated. Remove any residual liquid with a pipette.

    ⚠ *It is critical to completely remove all liquid from each well.*

18. Remove the plate off the magnetic separation device and add 50-100 µL of **MB Elution Buffer**. Pipette mix 20 times or vortex for 20 seconds.

19. Incubate the plate at 55°C for 5 minutes.

20. Place the sample plate back on the magnetic separation device and wait for 5 minutes or until the magnetic beads clear from the solution.

21. Transfer the eluate (cleared supernatant containing the DNA) to a new microplate for storage. Store the 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: US/Canada, +1 301-302-0144. Europe, +49 7250 33 13 403
Email: US/Canada, support@magbiogenomics.com. Europe, info.europe@magbiogenomics.com

Symptoms	Possible Causes	Comments
Low DNA yield	Poor cell lysis	Do not use more than 1 mL of high copy plasmid culture
		Cells may not be dispersed adequately prior to addition of SOL1/RNase A. Vortex cell suspension to completely disperse
		Increase incubation time with SOL2 to obtain a clear lysate
		SOL2 may not have been capped tightly. If not, it may need to be replaced
	Bacterial culture overgrown or not fresh	Do not incubate cultures for more than 16 hours 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 coincide with DNA yield on agarose gel	Trace contaminants eluted from the column increase the 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 the elution step

Ordering Information

HighPrep Plasmid DNA Plus Kit - DX

Catalog No.	Product	Description	Preps
HPPL-DP96E	HighPrep Plasmid DNA Plus Kit - DX (96 preps)	Magnetic bead-based kit for isolation of low/high copy plasmid DNA, BACs, PACs, Cosmids, and Fosmids	96
HPPL-DP96x4E	HighPrep Plasmid DNA Plus Kit - DX (384 preps)		384
HPPL-DP96x20E	HighPrep Plasmid DNA Plus Kit - DX (1,920 preps)		1,920
HPPL-DP96x40E	HighPrep Plasmid DNA Plus Kit - DX (3,840 preps)		3,840

Related Products

HighPrep PCR - DX

Catalog No.	Product
AC-60005E	HighPrep PCR - DX (5 mL)
AC-60050E	HighPrep PCR - DX (50 mL)
AC-60250E	HighPrep PCR - DX (250 mL)
AC-60500E	HighPrep PCR - DX (500 mL)

Magnetic Separation Devices

Catalog No.	Product
MYMAG-96	Handheld Magnetic Separation Device (96 well microplate format)
MYMAG-96X	Magnetic Separation Device (96 well ring format)
MBMS-12	MagStrip Magnet Stand (1.5 mL x 12)
MBMS-31550	15 mL and 50 mL Magnetic Stand Combo (3 x 15 mL and 3 x 50 mL)

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