EcoExtra™ Magnetic Beads Plasmid Kit Protocol

For research use only

Catalogue Number

MPD192, MPD576, MPD1920

Introduction

The EcoExtra™ Magnetic Beads Plasmid Kit was designed for high-throughput purification of plasmid DNA from up to 16 ml of cultured bacterial cells per reaction using an efficient magnetic beads system. A modified alkaline lysis method and RNase treatment are used to obtain clear cell lysate with minimal genomic DNA and RNA contaminants. Typical yields are 6-10 µg for high-copy number plasmid from 1 ml of cultured *E. coli* cells. The purified plasmid DNA is ready for use in restriction enzyme digestion, ligation, PCR, and sequencing reactions. **More important, the EcoExtra™ series kits enable up to 80% reduction of plastic material waste compared to silica-based kits. Make lab works a lot more eco-friendly.**

Components

	MPD006	MPD192	MPD576	MPD1920
MPD1 Buffer[1]	1 ml	25 ml	65 ml	220 ml
MPD2 Buffer[2]	1 ml	25 ml	75 ml	220 ml
MPD3 Buffer	1.5 ml	45 ml	100 ml	60 ml x1 240 ml x1
Wash Buffer[3] (Add ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)	50 ml x5 (200 ml x5)
Elution Buffer	1 ml	30 ml	75 ml	250 ml
RNase A (50 mg/ml)	Added	100 µl	260 µl	880 µl
MPD Magnetic Beads	100 µl	3 ml	9 ml	30 ml

1. Add provided RNase A to MPD1 Buffer then mix by shaking for a few seconds. Check the box on the bottle.
2. If precipitates have formed in MPD2 Buffer, warm in a 37ºC water bath, followed by gentle shaking to dissolve.
3. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Additional Requirements

- For 96 Well Plate Protocol: 96-well 2.2 ml deep well plate, 96-well 1.2 ml plate, 96-well 0.35 ml plate, magnetic separator for 96 well plates, centrifuge with 96-well plates swing bucket, orbital plate shaker, 37ºC incubator, isopropanol.
- For Single Tube Protocol: 1.5 ml centrifuge Tubes, magnetic separator for 1.5 ml centrifuge tube, 37ºC incubator, isopropanol.

96 Deep Well Plate Protocol

1. Grow Bacterial Cultures in a 96 Deep Well Plate

- Transfer 1.3 ml of fresh LB or 2X YT medium containing appropriate antibiotics into each well of a 96 Deep Well Plate then inoculate each well with a single plasmid containing *E. coli* bacterial colony.
- Seal the plate with microporous tape or adhesive film. When using non-porous adhesive tape, pierce 2-3 holes in the tape with a needle above each well to promote air exchange. Incubate at 37ºC for 20 hours with 180-250 rpm shaking. Do not allow culture blocks to overgrow.
- Pellet the bacterial culture in the plate by centrifugation for 10 minutes at 2,500 x g.
- Following centrifugation, remove the seal then remove the supernatant in each well by quickly inverting the plate. Blot the inverted block on a paper towel to remove excess media.

2. Resuspension

Add **100 µl of MPD1 Buffer (make sure RNase A was added)** to each well of the 96 Deep Well Plate then resuspend the cell pellet by pipette or vortex. Continue to pipette more than 20 times or until all traces of the cell pellet have been dissolved. Alternatively, vortex will take 2 to 3 minutes on a high setting. The cell pellet should be completely resuspended so that the mixture appears homogeneous and has no cell clumps.

3. Lysis

Add **100 µl of MPD2 Buffer** to each well of the 96 Deep Well Plate and gently mix by shaking or pipetting. Shake the deep well plate on an orbital shaker at 300-600 rpm for 5 minutes. Alternatively, gently pipette mix 2 times then incubate at room temperature for 5 minutes for complete lysis. Vigorous pipette mixing is not recommended. DO NOT incubate the sample longer than 10 minutes.

4. Neutralization

Add **150 µl of MPD3 Buffer** to each well of the 96 Deep Well Plate and gently mix by shaking or pipetting. Shake the deep well plate on an orbital shaker at 300-600 rpm for 5 minutes. Alternatively, pipette mix very gently for 3-5 times.

5. Cell Debris precipitation

Following neutralization, centrifuge the 96 Deep Well Plate at 4,700 ×g for 20 minutes to pellet the cell debris. If the pellet is not tightly bound to the bottom of the well, the centrifugation time should be increased.

6. DNA Binding

- Transfer 250 µl to 300 µl of the clear lysate to a clean 96-well 1.2 ml plate. Avoid touching and transferring any of the pelleted flocculent material for optimal results.
- Add **140 µl of Isopropanol** and **15 µl of MPD Magnetic Beads** (vortex magnetic beads to ensure they are in suspension) into the sample and mix the sample by pipetting for 10-15 times.
- Place the 96 Deep Well Plate to a magnetic separator (e.g. Geneaid 96 Magnetic Stand) to capture the MPD Magnetic Beads. Leave the plate on the magnetic separator for at least 5 minutes.
- Carefully aspirate and discard the supernatant without disturbing the MPD Magnetic Beads.
- Remove the 96 Deep Well Plate from the magnetic separator.

7. Wash

- Add **300 µl of Wash Buffer (make sure ethanol was added)** into the sample and mix the sample by pipetting for 10 times.
- Place the 96-well 1.2 ml plate to a magnetic separator to capture the MPD Magnetic Beads. Leave the plate on the magnetic separator for at least 2 minutes.
- Carefully aspirate and discard the supernatant without disturbing the MPD Magnetic Beads. Remove the 96 Deep Well Plate from the magnetic separator.
- Repeat the above wash steps once, for a total of two washes.

8. Elution

- Incubate the 96-well 1.2 ml plate at 37ºC for 5 minutes to evaporate the residue ethanol. DO NOT over dry the MPD Magnetic Beads.
- Add **25-100 µl of Elution Buffer** into the sample and mix the sample by pipetting for 10 times. Incubate the 96-well 1.2 ml plate at 37ºC for 5 minutes to fully elute the plasmid from the beads.
- Place the 96-well 1.2 ml plate to a magnetic separator to capture the MPD Magnetic Beads. Leave the plate on the magnetic separator for at least 2 minutes.
- Transfer the eluate (containing the purified plasmid) to a clean 96-well 0.35 ml plate, seal the plate with an adhesive film and store the plate at -20ºC.

Single Tube Protocol (for 1-4 ml cultured *E. coli* cells)

1. Cell Cultivation and Harvesting

Pick a single bacterial colony from a selective agar plate and inoculate the cell culture in 1-5 ml of LB medium containing appropriate antibiotics. Incubate at 37ºC for 12-16 hours with 150-180 rpm shaking. Transfer 1.5 ml of cultured bacterial cells to a 1.5 ml microcentrifuge tube. Centrifuge at 14-16,000 x g for 1 minute at room temperature to form a cell pellet then discard the supernatant completely. Repeat the harvesting step as required for samples between 1-4 ml using the same 1.5 ml microcentrifuge tube.

2. Resuspension

Add **100 µl of MPD1 Buffer (make sure RNase A was added)** to the 1.5 ml microcentrifuge tube containing the cell pellet. Resuspend the cell pellet completely by vortex or pipette until all traces of the cell pellet have been dissolved.

3. Lysis

Add **100 µl of MPD2 Buffer** to the resuspended sample then mix gently by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. Let stand at room temperature for at least 2 minutes to ensure the lysate is homogeneous. Do not exceed 5 minutes.

4. Neutralization

Add **150 µl of MPD3 Buffer** then mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. Centrifuge at 14-16,000 x g for 3 minutes at room temperature.

5. DNA Binding

- Transfer 250 µl to 300 µl of the clear lysate to a clean 1.5 ml centrifuge tube. Avoid touching and transferring any of the pelleted flocculent material for optimal results.
- Add **140 µl of Isopropanol** and **15 µl of MPD Magnetic Beads** (vortex magnetic beads to ensure they are in suspension) into the sample and mix the sample by pipetting for 10-15 times.
- Place the 1.5 ml centrifuge tube to a magnetic separator to capture the MPD Magnetic Beads. Leave the tube on the magnetic separator for at least 2 minutes.
- Carefully aspirate and discard the supernatant without disturbing the MPD Magnetic Beads.
- Remove the 1.5 ml centrifuge tube from the magnetic separator.

6. Wash

- Add **300 µl of Wash Buffer (make sure ethanol was added)** into the sample and mix the sample by pipetting for 10 times.
- Place the 1.5 ml centrifuge tube to a magnetic separator to capture the MPD Magnetic Beads. Leave the tube on the magnetic separator for at least 1 minutes.
- Carefully aspirate and discard the supernatant without disturbing the MPD Magnetic Beads. Remove the1.5 ml centrifuge tube from the magnetic separator.
- Repeat the above wash steps once, for a total of two washes.

7. Elution

- Incubate the 1.5 ml centrifuge tube at 37ºC for 5 minutes to evaporate the residue ethanol. DO NOT over dry the MPD Magnetic Beads.
- Add **25-100 µl of Elution Buffer** into the sample and mix the sample by pipetting for 10 times. Incubate the 1.5 ml centrifuge tube at 37ºC for 5 minutes to fully elute the plasmid from the beads.
- Place the 1.5 ml centrifuge tube to a magnetic separator to capture the MPD Magnetic Beads. Leave the tube on the magnetic separator for at least 2 minutes.
- Transfer the eluate (containing the purified plasmid) to a clean 1.5 ml centrifuge tube and store the sample at -20ºC.

Single Tube Protocol (for 5-8 ml, 9-12 ml, 13-16 ml of cultured *E. coli* cells)

1. Cell Cultivation and Harvesting

Pick a single bacterial colony from a selective agar plate and inoculate the cell culture in 1-5 ml of LB medium containing appropriate antibiotics. Incubate at 37ºC for 12-16 hours with 150-180 rpm shaking. Transfer 10-25 µl of cultured bacterial cells to 25 ml of LB medium containing appropriate antibiotics in a 100 ml erlenmeyer flask. Incubate at 37ºC for 12-16 hours with 150-180 rpm shaking. Transfer ■5-8 ml, ●9-12 ml ◆13-16 ml of cultured bacterial cells to a 50 ml centrifuge tube. Centrifuge at ≥3,000 x g for 10 minutes at room temperature to form a cell pellet then discard the supernatant completely.

2. Resuspension

Add **■200 µl**, **●300 µl**, **◆400 µl of MPD1 Buffer (make sure RNase A was added)** to the 50 ml centrifuge tube containing the cell pellet. Resuspend the cell pellet completely by vortex or pipette until all traces of the cell pellet have been dissolved. Transfer all MPD1 Buffer containing the resuspended cells to a 2.0 ml centrifuge tube.

3. Lysis

Add **■200 µl**, **●300 µl**, **◆400 µl of MPD2 Buffer** to the resuspended sample then mix gently by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. Let stand at room temperature for at least 2 minutes to ensure the lysate is homogeneous. Do not exceed 5 minutes.

4. Neutralization

Add **■300 µl**, **●450 µl**, **◆600 µl of MPD3 Buffer** then mix immediately by inverting the tube 10 times. Do not vortex to avoid shearing the genomic DNA. Centrifuge at 14-16,000 x g for 8 minutes at room temperature.

5. DNA Binding

- Transfer **■500 µl**, **●750 µl**, **◆1000 µl** of the clear lysate to a clean 2.0 ml centrifuge tube. Avoid touching and transferring any of the pelleted flocculent material for optimal results.
- Add **■280 µl**, **●420 µl**, **◆560 µl of Isopropanol** and **■30 µl**, **●45 µl**, **◆60 µl of MPD Magnetic Beads** (vortex magnetic beads to ensure they are in suspension) into the sample and mix the sample by pipetting for 10-15 times.
- Place the 2.0 ml centrifuge tube to a magnetic separator to capture the MPD Magnetic Beads. Leave the tube on the magnetic separator for at least 2 minutes.
- Carefully aspirate and discard the supernatant without disturbing the MPD Magnetic Beads.
- Remove the 2.0 ml centrifuge tube from the magnetic separator.

6. Wash

- Add **600 µl of Wash Buffer (make sure ethanol was added)** into the sample and mix the sample by pipetting for 10 times.
- Place the 2.0 ml centrifuge tube to a magnetic separator to capture the MPD Magnetic Beads. Leave the tube on the magnetic separator for at least 1 minutes.
- Carefully aspirate and discard the supernatant without disturbing the MPD Magnetic Beads. Remove the 2.0 ml centrifuge tube from the magnetic separator.
- Repeat the above wash steps once, for a total of two washes.

7. Elution

- Incubate the 2.0 ml centrifuge tube at 37ºC for 5 minutes to evaporate the residue ethanol. DO NOT over dry the MPD Magnetic Beads.
- Add **50-200 µl of Elution Buffer** into the sample and mix the sample by pipetting for 10 times. Incubate the 2.0 ml centrifuge tube at 37ºC for 5 minutes to fully elute the plasmid from the beads.
- Place the 2.0 ml centrifuge tube to a magnetic separator to capture the MPD Magnetic Beads. Leave the tube on the magnetic separator for at least 2 minutes.
- Transfer the eluate (containing the purified plasmid) to a clean 1.5 ml centrifuge tube and store the sample at -20ºC.

EcoExtra™ Magnetic Beads Plasmid Kit Test Data

Plasmid DNA was extracted using the EcoExtra™ Magnetic Beads Plasmid Kit from different volume of overnight *E. coli* (DH5 α) culturing in LB Broth, containing a 3 kb high copy plasmid pBluescript.

Culture volume	1 ml	2 ml	4 ml	8 ml	16 ml
Yield	6-10 µg	15-20 µg	26-32 µg	40-46 µg	70-78µg
$OD_{260/280}$	1.8 – 2.0				
$OD_{260/230}$	2.1-2.4				

Troubleshooting

Low Yield

Incomplete buffer and cell culture preparation.

- Add provided RNase A to MPD1 Buffer then mix by shaking for a few seconds. Check the box on the bottle then store at 2-8ºC for up to 6 months. If precipitates have formed in MPD2 Buffer, warm in a 37ºC water bath followed by gentle shaking to dissolve. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.
- We recommend using a single freshly isolated *E. coli* colony to inoculate into 1.3 ml of LB, 2X YT or TB medium. Solid and liquid medium should contain antibiotics. Do not use overgrown bacterial cultures (16-20 hours incubated in a 96 Deep Well Plate at 37ºC with 180-240 rpm shaking).

Culture growth medium was not removed completely.

Following centrifugation in the harvesting step, use a narrow pipette tip to ensure the supernatant is completely removed.

Cell pellet was not resuspended completely.

Resuspend the cell pellet completely by vortex or pipette. Continue to vortex or pipette until all traces of the cell pellet have been dissolved.

Bacterial cells were not lysed completely.

Following MPD2 Buffer addition, gently mix by shaking or pipetting then incubate at room temperature for 5 minutes.

Incorrect DNA Elution step.

After adding Elution Buffer, TE or water to the sample, mix the sample by pipetting for 10 times. If plasmid DNA are larger than 10 kb, use pre-heated Elution Buffer, TE, or water (60~70ºC) for elution. Incubate the sample at 37ºC for 5 minutes to fully elute the plasmid from the beads.

Eluted DNA Does Not Perform Well In Downstream Applications

Residual ethanol contamination.

Following the Wash Step, dry the MPD Magnetic beads in a 37ºC incubator for 5 minutes to evaporate the residue ethanol.

RNA contamination.

Add provided RNase A to MPD1 Buffer then mix by shaking for a few seconds. Check the box on the bottle then store at 2-8ºC for up to 6 months.

Genomic DNA contamination.

Do not use overgrown bacterial cultures. Use only fresh cultures as they will contain less genomic DNA than old cultures. During MPD2 and MPD3 Buffer addition, mix gently to prevent genomic DNA shearing.

Nuclease contamination.

Following the DNA Binding step, perform the additional Endonuclease Removal Wash Step. Add 300 µl of Endonuclease Removal Buffer (4.2M guanidine hydrochloride/40% isopropanol) into the sample and mix by pipetting for 10 times. Place the plate or tube to a magnetic separator to capture the MPD Magnetic Beads for at least 2 minutes. Carefully aspirate and discard the supernatant without disturbing the MPD Magnetic Beads. Remove the plate or tube from the magnetic separator then proceed with Wash Buffer addition.
NOTE: This optional wash step is recommended if using EndA+ bacterial strains such as *E.coli* HB 101, JM, or wild-type strains.