DNA-midi™ Plasmid DNA Purification Kit

iNtRON® Plasmid Kits (adjustable for both *endA*$^+$ and *endA*$^-$ strains)

DNA-midi™ Kit (CAT. 17251), midi scale	
M1 buffer	150 ml
M2 buffer	150 ml
M3 buffer	150 ml
RNase A sol. (10mg/ml)	8 ml
midi-Bead sol.	25 ml
Washing buffer A	35 ml
Washing buffer B (Add EtOH)	15 ml
Elution buffer	30 ml
Filter column	50 col.
Collection tube (2ml)	50 tubes

DNA-spin™ Kit (CAT. 17093), mini scale	
Sol I buffer	65 ml
Sol II buffer	65 ml
Sol III buffer	90 ml
RNase A sol. (10mg/ml)	3.5 ml
Washing buffer A	130 ml
Washing buffer B (Add EtOH)	50 ml
Columns	250 col.
Collection tube (2ml)	250 tubes

DNA-midi™ Data Information

Maximum recommended culture volumes

	DNA-midi™ Plasmid DNA Purification Kit	
	Midi protocol (500μℓ bead, one column)	Additional protocol (maxi) (< 2.0 ml bead, < four columns)
High-copy plasmids	25 ml medium	100 ml medium
Low-copy plasmids	100 ml medium	400 ml medium

This DNA-midi™ kit is adjustable for midi scale (midi protocol). However, you can extract massively plasmid DNA by using an additional protocol (maxi protocol). For the midi protocol, the expected yields are 75 ~ 150 ㎍ for high-copy plasmids and 25 ~ 150㎍ for low-copy plasmids. For the maxi protocol, the expected yields are 300 ~ 600㎍ for high-copy plasmids and 100 ~ 600㎍ for low-copy plasmids.

Recommended DNA-midi™ kit usage

High-copy plasmid (DNA yields)	25 ml ~ (75 ~ 150㎍)	50 ml ~ (150 ~ 300㎍)	75 ml ~ (225 ~ 450㎍)	~ 100 ml (300 ~ 600㎍)
Low-copy plasmid (DNA yields)	100 ml ~ (25 ~ 150㎍)	200 ml ~ (50 ~ 300㎍)	300 ml ~ (75 ~ 450㎍)	~ 400 ml (100 ~ 600㎍)
Used midi-Bead	500μℓ	1.0 ml	1.5 ml	2.0 ml
Used filter column	One filter col.	Two filter col.	Three filter col.	Four filter col.
Protocol	Midi protocol	Additional protocol (maxi protocol)		

Things to do before starting

▶ Add the provided RNase A (10mg/ml, spin down briefly before use) to M1 buffer before use, and then store at 4℃. Check M2 buffer for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37℃. Add 60ml EtOH (100% ethanol) to Washing buffer B before use.

▶ Mix thoroughly the midi-Bead before use. The midi-Bead has been developed directly by iNtRON (patent pending). The midi-Bead is very solid pellet, because the midi-Bead is a chemical-based fiber bead. So, before use, you have to vigorously mix the glass bottle up and down.

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DNA-midi™ characteristics

① This midi-Bead is very fine chemical beads. For this reason, the midi-Bead can be eluted a little with buffers in washing step and elution step. However, the eluted midi-Bead does not make any effects for DNA yields and purity. So, do not care for midi-Bead elutes.
② The DNA-midi™ kit's protocol is suitable for both *endA*$^+$ (endonuclease A$^+$) and *endA*$^-$ *E. coli* strains.
③ Easy to use – organic extraction or ethanol precipitation is not required.
④ Save time - takes only 50 ~ 60 minutes to extract plasmid DNA.
⑤ No phenol or chloroform is used.
⑥ Minimal nicks of plasmid DNA guarantees good results in plasmid DNA sequencing, transfection, restriction enzyme digestion, *in vitro* transcription/translation, or etc.

Plasmid size

Plasmids up to approximately 20 ~ 30 Kb can be efficiently purifed using DNA-midi™ purification protocols. However, plasmids larger than 35 ~ 40 Kb, may exhibit a little reduced DNA yields. Prewarming the elution buffer to 50℃ may help to increase the yield of large plasmids.

Storage conditions

The iNtRON DNA-midi™ kit should be stored at room temperature (15 ~ 25℃). After adding RNase A, M1 buffer should be stored at 2 ~ 8℃ and is stable for 1 years. Other buffers and RNase A stock solution can be stored for more than 1 year at room temperatures (15 ~ 25℃). The expired date is described on a box.

Product use limitations

The iNtRON plasmid kits are developed, designed and sold for research use only. They are not to be used for human diagnostic or drug purposes or to be administered to humans.

Safety information

Always wear a suitable gown, gloves, and protective goggles when working with chemicals. For more details, please consult the appropriate materials safety data sheet (MSDS). If you need MSDS, we will send you a FAX. Emergency medical information can be obtained from Customer Center, Korea (+82-31-730-7280).

DNA concentration and yields according to Elution buffer amounts

DH5α (25ml, 500μℓ midi-Bead) containing EGFP plasmid DNA, OD_{600} = 1.67

Elution buffer	OD_{260}	OD_{280}	$OD_{260/280}$	Concentration	Total DNA yields
100 μℓ	1.372	0.743	1.82	1372 ng/μℓ	137 ㎍
200 μℓ	1.272	0.711	1.79	711 ng/μℓ	143 ㎍
500 μℓ	0.541	0.290	1.86	290 ng/μℓ	148 ㎍

You can elute DNA by adding an appropriate Elution buffer per one filter column. We recommend you to add 300 ~ 500μℓ Elution buffer per one filter column for 300 ~ 500ng/μℓ DNA concentration.

Midi Protocol : *Plasmid DNA Purification Using Midi Protocol*

This protocol is designed for preparation of up to 150㎍ of high- or low-copy plasmid DNA by using midi protocol. Also, you can extract plasmid DNA up to 600㎍ with maxi protocol.

1. **Inoculate 25 ml medium for high-copy plasmids (100 ml medium for low-copy plasmids), and then grow at 37℃ for 12 ~ 16 hrs with vigorous shaking (OD_{600} = 1.0 ~ 1.5).**
2. **Harvest the bacterial cells by centrifugation at maximum speed (13,000rpm) for 5 min at 4℃.** You may progress this harvest step according to your lab conditions. After harvesting, remove all traces of supernatant by inverting the open centrifuge tube until all medium has been drained.

3. **Resuspend the bacterial pellet in 3 ml of M1 buffer (Add RNase A before use).**
   The bacteria should be resuspended completely by vortexing vigorously until no cell clumps remain.
4. **Add 3 ml of M2 buffer, mix gently by inverting 10 times, and incubate at RT for 5 min.**
   Do not vortex, as this will result in shearing of genomic DNA. The lysate should appear viscous. Do not allow the lysis reaction to proceed for more than 5 min.
5. **Add 3 ml of M3 buffer, mix gently by inverting 10 times, and incubate on ice for 15 ~ 20 min.**
   After addition of M3 buffer, a white material forms and the lysate becomes less viscous.
6. **Centrifuge at maximum speed (13,000rpm) for 10 min at 4℃. Transfer supernatant into new tube.**
   After centrifugation, the supernatant should be clear. However, if the supernatant is not clear, please centrifuge again (optional : 2nd centrifugation step).
7. **Add 500㎕ of midi-Bead solution into the upper supernatant, and mix gently (Do not vortex !).**
   The midi-Bead pellet is very solid, because it is a chemical-based bead. You should mix up and down vigorously before use.
8. **Centrifuge at maximum speed (13,000rpm) for 5 ~ 10 min at 4℃ for getting midi-Bead pellet.**
   Decant supernatant gently. Remove all traces as soon as possible by inverting the open centrifuge tube. Do not allow the centrifugation step to proceed for more than 10 min.
9. **Add 700㎕ of Washing buffer A, and resuspend midi-Bead with gently pipetting (Do not vortex !).**
   The resuspension of midi-bead by pipetting could be not convenient because of its clumps. The midi-Bead may be resuspended a little just to the extent that you can pipette easily.
10. **Transfer the resuspended midi-Bead solution onto the Filter column, and spin-down (13,000rpm at RT) for 30 sec.**
    Cut the bottom of Filter column with a hand. If not necessary, you may not close the cap of Filter column. When spin-down, the midi-Bead (very fine chemical bead) could be eluted a little in a collection tube. Although the midi-Bead is eluted somewhat, there is no effect in DNA yield and purity.
11. **Add 700㎕ of Washing buffer B, and spin-down for 30 sec at RT (two times).**
    Do not resuspend the midi-Bead with Washing buffer B ! Just add and proceed spin-down step as same as mini column kits. Please be careful for not touching the midi-Bead with a pipette tip. After spin-down, carry out Washing buffer B step again. This 2nd step is for getting high purity of plasmid DNA.
12. **Discard the elute, and spin-down for additional 1 min to dry the midi-Bead. Then, transfer the air-dried Filter column onto new 1.5ml tube.**
    Completely remove the residual ethanol. The residual EtOH may affect the purity of plasmid DNA.
13. **Add 300 ~ 500㎕ of Elution buffer, and after incubating at RT for 1 min, spin-down for 1 min.**
    You can concentrate plasmid DNA by adding 100 ~ 200㎕ of Elution buffer (refer to page 1). And more, prewarming the elution buffer to 50?C may help to increase the yield of large plasmids. After spin-down, you could see very small white pellet (midi-Bead) in the bottom of 1.5ml tube.
14. **Centrifuge again at 13,000 rpm for 5 min, and transfer gently the supernatant into new 1.5ml tube.**
    When you transfer the supernatant, please be careful for not pipetting the midi-Bead. To determine the yield, DNA concentration should be determined by both UV spectrophotometer and quantitative analysis on an agarose gel.

Maxi Protocol : Plasmid DNA Purification Using Maxi Protocol

This protocol is an additional protocol in DNA-midi™ kit. This additional maxi protocol is designed for preparation of up to 600㎍ of high- or low-copy plasmid DNA. For more detail information, please refer to midi protocol. When you use this maxi protocol, please be sure to read the midi protocol in advance.

1. **Inoculate 50 ml, 75 ml, or 100 ml medium for high-copy plasmids (200 ml, 300 ml, or 400 ml for low-copy plasmids), and then grow at 37℃ for 12 ~ 16 hrs with vigorous shaking (OD_{600} = 1.0~1.5).**

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2. **Harvest the bacterial cells by centrifugation at maximum speed (13,000rpm) for 5 min at 4℃.**
3. **Resuspend the bacterial pellet in 6 ml, 9 ml, or 12 ml of M1 buffer (Add RNase A before use).**
4. **Add 6 ml, 9 ml, or 12 ml of M2 buffer, mix gently by inverting 10 times, and incubate at room temperature for 5 min.**
5. **Add 6 ml, 9 ml, or 12 ml of M3 buffer (neutralization buffer), mix gently by inverting 10 times, and incubate on ice for 15 ~ 20 min.**
6. **Centrifuge at maximum speed (13,000rpm) for 10 min at 4℃. Transfer supernatant into new tube.**
7. **Add 1.0 ml, 1.5 ml, or 2.0 ml of midi-Bead solution into the upper supernatant, and mix gently.**
8. **Centrifuge at maximum speed (13,000rpm) for 5 ~ 10 min at 4℃ for getting midi-Bead pellet.**
9. **Add 1.4 ml, 2.1 ml, or 2.8 ml of Washing buffer A, and resuspend midi-Bead with gently pipetting.**
10. **Transfer the resuspended midi-Bead solution equally (each 700 ㎕) onto two, three, or four of Filter columns, and spin-down (13,000rpm at RT) for 30 sec.**
11. **Add 700㎕ of Washing buffer B into each tubes, and spin-down for 30 sec at RT (two times).**
12. **Discard the elute, and spin-down for additional 1 min to dry the midi-Bead. Then, transfer the air-dried Filter column onto new 1.5ml tube.**
13. **Add 300 ~ 500㎕ of Elution buffer into each tubes, and after incubating at RT for 1 min, spin-down for 1 min.**
14. **Centrifuge again at 13,000 rpm for 5 min, and transfer gently the supernatant into new 1.5ml tube.**
    To determine the yield, DNA concentration should be determined by both UV spectrophotometer and quantitative analysis on an agarose gel.

General information for plasmid DNA

For more detailed information, please refer to the manual guide of our DNA-spin™ (CAT. 17093, mini column). If you need, please do not hesitate to contact us (T. +82-505-550-5600, intronbio@intronbio.com).

Troubleshooting guide

Problem	Possible Cause	Recommendation
Low or no yield	Plasmid did not propagate	Check that the conditions for optimum growth were met.
	M2 buffer is precipitated	Check the M2 buffer for SDS precipitation due to low storage temperature and dissolve SDS by warming to 37ºC.
	M2 buffer incompletely mixed	Ensure complete mixing all buffers. When put and mix M2 buffer and M3 buffer, do not mix strongly.
	Cell resuspension incomplete	Pelleted cells should be completely resuspended in M1 buffer. Do not add M2 buffer until an even suspension is obtained.
	Wrong reagent used	Add 100% EtOH to Washing buffer B before use.
RNA in the eluate		M1 buffer should be stored at 4?C after adding RNase A. Check the culture volume against recommended volumes. If necessary, add more RNase A.
DNA is nicked /sheared/degraded	Endonuclease-containing host	Proceed Washing buffer A step.
Genomic DNA in the eluate	Lysis time was too long	When add M2 buffer and M3 buffer, do not shake strongly. Ensure that the lysis step does not exceed 5min.

If you have any questions or experience any difficulties regarding any aspect of iNtRON products in general, please do not hesitate to contact us.