ExtractNow™ Plasmid Mini Kit

Extraction of high-copy and low-copy plasmid DNA, P1 constructs, etc.

INSTRUCTIONS FOR USE

FOR USE IN RESEARCH AND QUALITY CONTROL

Symbols

Lot No.

Cat. No.

Expiry date

Storage temperature

Number of reactions

Manufacturer

INDICATION

The ExtractNow™ Plasmid Mini Kit is a very efficient tool for isolation and purification of plasmid DNA (pDNA) from bacterial suspensions and cultures. The kit facilitates the isolation of high and low copy pDNA as well as P1 constructs with a maximum yield of up to 40 µg pDNA.
For high copy plasmid DNA, 0.5 to 5 ml bacterial overnight culture are recommended as starting material, whereas 5 to 10 ml is the recommended volume range for low copy plasmid DNA or P1 constructs. The purified pDNA is free of prokaryotic contaminants, highly pure with a OD260/280 ratio of 1.8-2.0 and can be used immediately for many downstream applications such as sequencing or transfection. The average yield from a 2 ml bacterial culture containing a high-copy plasmid is between 6 and 20 µg.

TEST PRINCIPLE

The method is simple and consists of five general steps: (1) pelleting and resuspending bacterial cells, (2) alkaline lysis, (3) precipitation of bacterial chromosomal DNA and proteins (4) binding of pDNA to a filter membrane, and (5) elution of purified pDNA by low salt buffer.
The procedure does not require phenol/chloroform and, thanks to the spin-colum-based approach, needs minimal handling time. The kit's chemistry enables fast purification in about 25 minutes.

CONTENT

Each kit contains reagents for 10 or 50 extractions. The expiry date of the unopened package is given on the label. Store all components at room temperature (18 to 25 °C). Before each use, equilibrate all components at room temperature and dissolve any precipitates that may have formed in the buffers by moderate warming.

Kit Component	Quantity	
	10 extractions Cat. No. 605-1010	**50 extractions Cat. No. 605-1050**
Spin columns (orange)	10 units	50 units
Collection tubes	10 units	50 units
Resuspension Buffer	12 ml	30 ml
Lysis Buffer A	15 ml	30 ml
Neutralizer	12 ml	32 ml
Wash Buffer A1	15 ml	30 ml
Wash Buffer A2	6 ml (add 9 ml ethanol (>96%) before first use)	20 ml (add 30 ml ethanol (>96%) before first use)
Elution Buffer B	2 ml	15 ml

The lot-specific quality control certificate (Certificate of Analysis) can be downloaded from our website (www.minerva-biolabs.com / www.minervabiolabs.us).

USER-SUPPLIED CONSUMABLES AND EQUIPMENT

The ExtractNow™ Plasmid Mini Kit contains reagents for extraction and purification of plasmid DNA from bacterial cultures. Additional consumables and equipment are supplied by the user:

- Ethanol > 96 % abs. (molecular biology grade)
- 1.5 ml and 2 ml reaction tubes and 15 ml tubes
- Microcentrifuge for 1.5 ml, 2.0 ml or 15 ml reaction tubes
- Heat block or thermomixer for 1.5 ml and 2 ml reaction tubes
- Pipettes with corresponding filter tips (10 and 1000 μl)

SPECIMEN

Best results are obtained with 0.5 ml to 10 ml of bacterial cultures properly grown overnight. Notably, it is important not to overload the spin columns with larger volumes to avoid purification performance loss.
The typical volumes of starting material are:

- 0.5 to 5 ml of bacterial suspension for high copy plasmid DNA (see protocol 1)
- 5 to 10 ml of bacterial suspension for low copy plasmid DNA or P1 constructs (see protocol 2)

PRECAUTIONS

The ExtractNow™ Plasmid Mini Kit is for research use only. The kit should be used by trained laboratory staff only.

Always wear suitable lab coat, disposable gloves, and protective goggles.

In case of contact, flush eyes or skin with water. Do not swallow components of the kit. Clean with suitable laboratory detergent and water, if any liquid is spilt.

This kit can be disposed of as municipal waste according to local guidelines.

IMPORTANT NOTES

⇒ Set up the temperature of the heat block to 50 °C.

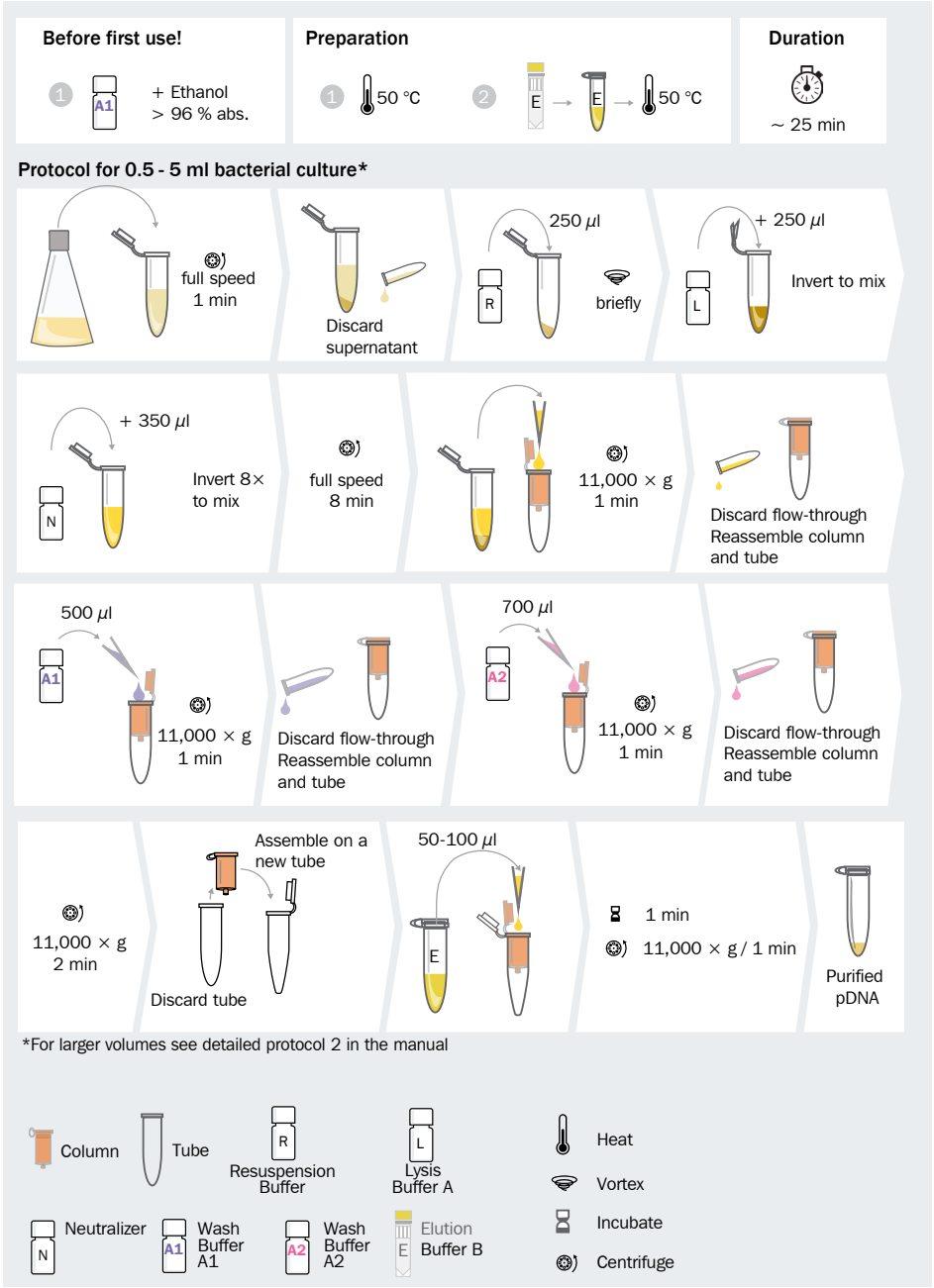
⇒ Pre-warming the Elution Buffer B to 50 °C will increase the DNA yield.

⇒ Ensure that ethanol was added to Wash Buffer A2. Do not use any alcohol other than ethanol, as it will lead to inconsistent yields.

⇒ The centrifugation steps should be carried out at room temperature. The reagents supplied should not be mixed with reagents from different lots but used as an integral unit. The reagents of the kit must not be used beyond the expiry date.

⇒ Follow the exact protocol. Any deviation may affect the results.

PROCEDURE - OVERVIEW



PROCEDURE - STEP BY STEP

⇒ Before first use, reconstitute Wash Buffer A2 with absolute ethanol.
⇒ Set the temperature of the heat block to 50 °C.
⇒ Pre-warm needed volume of Elution Buffer B to 50 °C for better elution results.
⇒ If the Lysis Buffer A shows any precipitates, dissolve by moderate warming and equilibrate at room temperature, before application.

Protocol 1: Isolation of plasmid DNA from 0.5 to 5 ml bacterial overnight culture

1.1	Transfer 0.5 ml to 5 ml of bacterial suspension to a 1.5 ml, 2 ml, or 15 ml reaction tube. Pellet cells by centrifugation at max. speed for 1 min and discard the supernatant.
1.2	Add 250 µl Resuspension Buffer to the cell pellet and mix it by vortexing or pipetting up and down several times until the suspension appears homogenous. Note: If a 15 ml reaction tube was used, transfer the re-suspended sample into a fresh 1.5 or 2 ml reaction tube and proceed to the next step.
1.3	Add 250 µl Lysis Buffer A and mix by inverting several times. Do not vortex to avoid contamination with sheared chromosomal DNA. Do not extend the lysis step for longer than 5 min as further incubation will be detrimental for DNA integrity.
1.4	Add 350 µl Neutralizer and mix carefully by inverting 8 times. Centrifuge at full speed for 8 min. Transfer the supernatant to a spin column placed on a collection tube.
1.5	Centrifuge at 11,000 × g for 1 min, discard flow-through and re-assemble column and tube.
1.6	Add 500 µl Wash Buffer A1 to the spin column and centrifuge at 11,000 × g for 1 min. Discard flow-through and re-assemble spin column and collection tube.
1.7	Repeat wash step with 700 µl reconstituted Wash Buffer A2. Re-assemble spin column and collection tube.
1.8	Centrifuge at 11,000 × g for 2 min to remove all traces of ethanol. Discard the collection tube and place the spin column on a new 1.5 ml tube.
1.9	Add 50 to 100 µl pre-warmed Elution Buffer B directly on the filter membrane and incubate at room temperature for 1 min. Centrifuge at 11.000 × g for 1 min. Note: The elution volume should be adjusted according to the expected pDNA yield. Use smaller volumes in two subsequent centrifugation steps to increase the plasmid DNA yield. Store the extracted plasmid DNA at +2 - +8 °C or at ≤ –18 °C for long-term storage.

Protocol 2: Isolation of plasmid DNA from 5 to 10 ml bacterial culture

2.1	Transfer 5 ml to 10 ml of bacterial suspension to a 15 ml tube. Pellet cells by centrifugation at max. speed for 1 min and discard the supernatant.
2.2	Add 500 µl Resuspension Buffer to the cell pellet and mix it by vortexing or pipetting up and down several times until the suspension appears homogenous. Transfer the re-suspended sample into a fresh 2 ml reaction tube and proceed to the next step.
2.3	Add 500 µl Lysis Buffer A and mix by inverting several times. Do not vortex to avoid contamination with sheared chromosomal DNA. Do not extend the lysis step more than 5 min as further incubation will be detrimental for DNA integrity.
2.4	Add 600 µl Neutralizer and mix carefully by inverting 8 times. Centrifuge at full speed for 10 min and transfer 700 µl supernatant in a spin column placed on a collection tube.
2.5	Centrifuge at 11,000 × g for 1 min and discard the flow-through. Re-assemble spin column and collection tube. Transfer the remaining sample volume and centrifuge again until the entire volume has passed through the filter membrane.
2.6	Add 500 µl Wash Buffer A1 to the spin column and centrifuge at 11,000 × g for 1 min. Discard flow-through and re-assemble spin column and collection tube.
2.7	Repeat wash step with 700 µl reconstituted Wash Buffer A2. Re-assemble spin column and collection tube.
2.8	Centrifuge at 11,000 × g for 2 min to remove all traces of ethanol. Discard the collection tube and place the spin column on a new 1.5 ml tube.
2.9	Add 50 to 100 µl pre-warmed Elution Buffer B directly on the filter membrane and incubate at room temperature for 1 min. Centrifuge at 11.000 × g for 1 min. Note: The elution volume should be adjusted according to the expected pDNA yield. Use smaller volumes in two subsequent centrifugation steps to increase the plasmid DNA yield. Store the extracted plasmid DNA at +2 - +8 °C or at ≤ –18 °C for long-term storage.

APPENDIX

Limited Product Warranty

This warranty limits our liability for replacement of this product. No warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided. Minerva Biolabs shall have no liability for any direct, indirect, consequential, or incidental damages arising from of the use, the results of use, or the inability to use this product.

Trademarks

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Related Products

DNA Extraction kits		
56-1010/1050/1200	Venor®GeM Sample Preparation Kit	10/50/200 extractions
601-1010/1050/1200	ExtractNow™ DNA Mini Kit	10/50/200 extractions
602-1010/1050/1200	ExtractNow™ Blood DNA Mini kit	10/50/200 extractions
603-1010/1050/1200	ExtractNow™ RNA Mini kit	10/50/200 extractions
604-1010/1050/1200	ExtractNow™ Cleanup kit	10/50/200 extractions
606-1010/1050/1200	ExtractNow™ Virus DNA/RNA kit	10/50/200 extractions
607-1010/1050	ExtractNow™ Vegan Control	10/50 extractions
608-1010/1050	ExtractNow™ Meat ID	10/50 extractions
PCR Mix		
191-0025/-0100/-0250	ConviFlex™ DNAmp Mix, with Taq polymerase for conventional and qPCR	25/100/250 reactions
192-0025/-0100/-0250	ConviFlex™ RT-Taq Mix, with retrotrascriptase and Taq polymerase for conventional RT-PCR and RT-qPCR	25/100/250 reactions
MB Taq Polymerase		
53-0050/-0100/-0200/-0250	MB Taq DNA Polymerase (5 U/µl)	50/100/200/250 units
53-1050/-1100/-1200/-1250	MB Taq DNA Polymerase (1 U/µl)	50/100/200/250 units
PCR Cycler Validation		
57-2102/-2103	PCR Cycler Check™ Advance/OneStep	6 strips, 8 vials each/100 reactions
57-2202	qPCR Cycler Check™	100 reactions
Lab Monitoring Kits		
181-0010/-0050	SwabUp™ Lab Monitoring, For sample collection and DNA extraction	10/50 samples
Contamination Control Kits for conventional PCR		
11-7024/-7048/-7096/-7240	Venor®GeM Advance Mycoplasma Detection Kit	24/48/96/240 reactions
11-8025//-8100/-8250	Venor®GeM OneStep Mycoplasma Detection Kit	25/100/250 reactions
12-1025/-1100/-1250	Onar® Bacteria Detection Kit	25/100/250 reactions
Contamination Control Kits for qPCR		
11-9025/-9100/-9250	Venor®GeM qEP Mycoplasma Detection Kit	25/100/250 reactions
11-91025/-91100/-91250	Venor®GeM qOneStep Mycoplasma Detection Kit	25/100/250 reactions
PCR Clean™		
15-2025/-2200	DNA Decontamination Reagent, spray bottle/refill canister	250 ml/4×500 ml
15-2500	DNA Decontamination Reagent, refill canister	5 l
15-2001	DNA Decontamination Reagent, Wipes in dispenser box	50 wipes
15-2002	DNA Decontamination Reagent, Wipes, refill pack	5×50 wipes
LabClean™		
15-4100	Molecular Microbiology Lab Cleaner	1 l
Mycoplasma Off™		
15-1000/-5000	Surface Disinfectant Spray, spray bottle, refill canister	1 l/5 l
15-1001	Surface Disinfectant Wipes in dispenser box	50 wipes
15-5001	Surface Disinfectant Wipes in refill pack	5×50 wipes
WaterShield™		
15-3015/3020/3050	Water Disinfection Additive for incubators and water baths, 200× concentrate	15×10 ml/3×50 ml/500 ml
ZellShield®		
13-0050/-0150	Cell culture contamination preventive 100× concentrate	50 ml/ 3×50 ml
qPCR Kits for Food Contamination Testing		
36X-X025	Food Control™ qPCR	25 reactions
360-1025	Salmonella enterica	25 reactions

360-2025	Yersinia enterocolitica	25 reactions
360-3025	Shigella spp.	25 reactions
360-4025	Campylobacter spp.	25 reactions
360-5025	Clostridium perfringens	25 reactions
360-6025	Shiga Toxin 1	25 reactions
360-7025	Shiga Toxin 2	25 reactions
360-8025	Escherichia coli O157	25 reactions
360-9025	Escherichia coli O104	25 reactions
361-1025	Listeria spp.	25 reactions
361-2025	Listeria monocytogenes	25 reactions
361-3025	Salmonella spp.	25 reactions

qPCR Kits for Water Contamination Testing

33-2025/-2100/-2250	AquaScreen® Legionella species	25/100/250 reactions
34-2025/-2100/-2250	AquaScreen® Legionella pneumophila	25/100/250 reactions
34-6025/-6100/-6250	AquaScreen® Pseudomonas aeruginosa	25/100/250 reactions
34-7025/-7100/-7250	AquaScreen® Escherichia coli	25/100/250 reactions

qPCR Kits for Vegan Control

370-2025/-2100	Vegan Control™	25/100 reactions

qPCR Kits for Meat Identification

370-1025/-1100	Meat ID™ Halal	25/100 reactions

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