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.

Order 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 suspension. The kit facilitates isolating 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 is recommended as starting material, whereas 5 to 10 ml is usually used for low copy plasmid DNA or P1 constructs. The purified pDNA is free of prokaryotic contaminants, highly pure with an average OD260/280 ratio of 1.8 to 2.0 and can be used immediately for many downstream applications such as sequencing.

PRINCIPLE OF THE METHOD

The method is simple and consists of five general steps: (1) pellet and re-suspend 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 extraction and needs minimal handling time. The kit's chemistry facilitates fast purification in about 25 minutes.

CONTENT

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

Kit component	10 extractions (605-1010)	50 extractions (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 QC certificate (*Certificate of Analysis*) can be downloaded from our website (www.minerva-biolabs.com).

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 is supplied by the user:

- Ethanol > 96 % abs.
- 1.5 ml, 2.0 ml reaction tubes and 15 ml tubes (optional)
- Microcentrifuge for 1.5 ml, 2.0 ml or 15 ml reaction tubes (optional)
- Heat block or thermomixer for 1.5 ml or 2.0 ml
- Pipettes with corresponding filter tips (100 and 1000 µl)

SPECIMEN

Best results are obtained with volumes of 0.5 ml to 10 ml of bacterial cultures grown overnight. It is also important not to overload spin columns. 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).

The average yield from a 2 ml bacterial culture containing a high-copy plasmid is between 6 and 20 µg.

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 heat block to 50 °C. Pre-warmed Elution Buffer B to 50 °C will increase the DNA yield.
- Ensure that ethanol was added to Wash Buffer A2. Do not use other alcohol apart from 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 LOT but used as an integral unit. The reagents of the kit must not be used beyond shelf life.

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

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 the use, the results of use, or the inability to use this product.

ExtractNow™ Plasmid Mini Kit

Included

= = R Resuspension Buffer L Lysis Buffer A

N Neutra-lizer A1 Wash Buffer A1 A2 Wash Buffer A2 E Elution Buffer B

Duration

~ 25 min

Additionally required

- Ethanol > 96 % abs.
- 1.5 ml or 2 ml or 15 ml Reaction tubes
- Tools: Microcentrifuge
  Thermomixer
  Vortexer
  Pipettes + tips

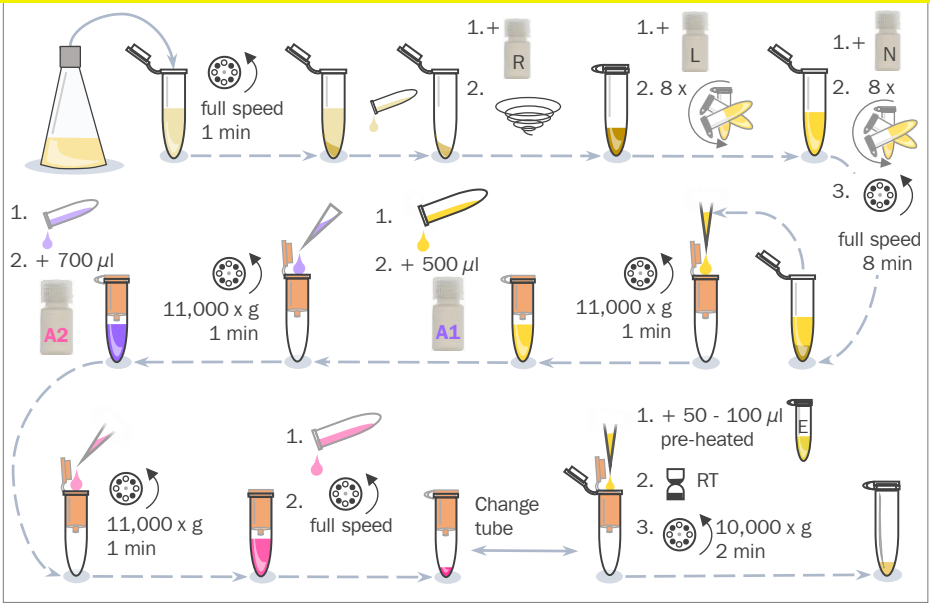
Before first use!

1 A2 + Ethanol > 96 % abs.

Preparation

1 50 ° 2 E E 50 °

Procedure



Storage

- Store kit components at room temperature (18-25 °C). The expiry date of the unopened package is marked on the package label.
- Store purified DNA for 1 week at +2-8 °C or at -18 °C for long term storage.

Legend

Cell suspension
Buffer A1
Buffer A2
Buffer E
Purified DNA

Heat
Vortex
Incubate
Centrifuge

PROCEDURE - STEP BY STEP

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

⇒ Before first use reconstitute Wash Buffer A2 with absolute ethanol
⇒ Set 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, resolve sediments by moderate warming. For subsequent application the solution must be at room temperature.

1.1	Transfer 0.5 ml to 5 ml of your bacterial suspension into a 1,5 ml or 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 with 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 more than 5 min as further incubation will be detrimental for DNA integrity.
1.4	Add 350 µl Neutralizer and mix by inverting 8 times carefully. Centrifuge at full speed for 8 min. Transfer the supernatant into a spin column placed in a collection tube.
1.5	Centrifuge at 11,000 x 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 x 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 x g for 2 min to remove all traces of ethanol. Discard the collection tube and place the spin column in 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 x g for 1 min. Note: The elution volume should be adjusted with respect 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 4 °C or at –20 °C for long time storage.

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

⇒ Before first use reconstitute Wash Buffer A2 with absolute ethanol
⇒ Set 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, resolve sediments by moderate warming. For subsequent application the solution must be at room temperature.

2.1	Transfer 5 to 10 ml of your bacterial suspension into 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 resuspended sample into a fresh 2 ml reaction tube and proceed with 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 by inverting 8 times carefully. Centrifuge at full speed for 10 min and transfer 700 µl supernatant into a spin column placed in a collection tube.

2.5	Centrifuge at 11,000 x 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 x 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 x g for 2 min to remove all traces of ethanol. Discard the collection tube and place the spin column in 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 x g for 1 min. Note: The elution volume should be adjusted with respect 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 4 °C or at –20 °C for long time storage.

Related Products

DNA/RNA Extraction kits

56-1010/1050/1200	Venor®GeM Sample Preparation Kit	10/50/200 extractions
56-2096	Venor®GeM Sample Preparation Kit - IP C16	96 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
605-1010/1050/1200	ExtractNow™ Plasmid Mini kit	10/50/200 extractions
606-1010/1050/1200	ExtractNow™ Virus DNA/RNA kit	10/50/200 extractions

MB *Taq* DNA 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

Contamination Control PCR kits

11-1025/1050/1100/1250	Venor®GeM Classic Mycoplasma Detection Kit	25/50/100/250 tests
11-7024/7048/7096/7240	Venor®GeM Advance Mycoplasma Detection Kit	24/48/96/240 tests
11-8025/8050/8100/8250	Venor®GeM OneStep Mycoplasma Detection Kit	25/50/100/250 tests
12-1025/1050/1100/1250	Onar® Bacteria Detection Kit	25/50/100/250 tests
11-9025/9100/9250	Venor®GeM qEP Mycoplasma Detection Kit	25/100/250 tests

Mycoplasma Elimination

10-0200/0500/1000	Mynox® Mycoplasma Elimination Reagent	2/5/10 treatments
10-0201/0501/1001	Mynox® Gold Mycoplasma Elimination Reagent	2/5/10 treatments

PCR Quantification Standards, 1x10^8 genomes / vial

52-0112	*Mycoplasma orale*
52-0115	*Mycoplasma gallisepticum*
52-0116	*Acholeplasma laidlawii*
52-0117	*Mycoplasma fermentans*
52-0119	*Mycoplasma pneumonia*
52-0124	*Mycoplasma synoviae*
52-0129	*Mycoplasma arginini*
52-0130	*Mycoplasma hyorhinis*
52-0164	*Spiroplasma citri*

See Minerva homepage for further available species

PCR Clean™ (formerly DNA Remover™)

15-2025/2200	DNA Decontamination Reagent, spray bottle/refill bottles	250 ml/4x 500 ml
15-2201	Wipes	120 wipes in a dispenser box
15-2202	Wipes, refill packs	5 x 120 wipes in a bag
15-2203	Wipes, single wrapped	30 wipes

Mycoplasma Off™

15-1000	Surface Disinfectant Spray, spray bottle	1000 ml
15-5000	Surface Disinfectant Spray, refill bottles	5 x 1000 ml
15-1001	Surface Disinfectant Wipes in dispenser box	120 wipes
15-5001	Surface Disinfectant Wipes, refill pack	5 x 120 wipes
15-1030	Wipes, single wrapped	30 sachets

ZellShield™

13-0050/0150	Contamination Prevention Reagent 100x concentrate	1000 ml/ 5 x 1000 ml

WaterShield™

15-3025/3075	Water Disinfection Additive for incubators and water baths 200x concentrate	30 x 5 ml/500 ml

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