Article number:
11003321 (250 pcs.)

Version: 062018

Research use only

Axonlab LifeScience
GENOMICS

extrAXON
PLASMID MINI KIT

Kit for isolation of plasmid DNA

The **extrAXON PLASMID MINI KIT** is designed for a rapid and efficient purification of high quality plasmid DNA from recombinant *Escherichia coli* strains. The isolation protocol and buffer formulations were optimized for high isolation efficiency and DNA purity. The product is intended for research use only.

Axonlab
connecting ideas

www.axonlab.com

extrAXON
PLASMID MINI KIT

Instructions to use
Version 062018

I. | Intended Use

The **extrAXON PLASMID MINI KIT** is designed for a rapid and efficient purification of high quality plasmid DNA from recombinant *Escherichia coli* strains. The isolation protocol and buffer formulations were optimized for high isolation efficiency and DNA purity. The product is intended for research use only.

II. | COMPONENTS OF THE KIT AND STORAGE CONDITIONS

Number of isolation	250 isolations	Storage Conditions
Resuspension Buffer	63 ml	15°C – 25°C[1]
▶ RNaseA* (lyophilized)	1 tube	+4°C
BalticBlue	1 tube	15°C – 25°C
Lysis Buffer	63 ml	15°C – 25°C
Neutralization Buffer	88 ml	15°C – 25°C
NW Buffer	125 ml	15°C – 25°C
Wash Buffer** (conc.)	42 ml	15°C – 25°C
Elution Buffer	5 x 10 ml	15°C – 25°C
DNA Purification Columns placed in Collection Tubes	5 x 50 pcs	15°C – 25°C
Loading Buffer	1 tube	15°C – 25°C

[1] After adding RNaseA, **Resuspension Buffer** should be kept at +4°C.

* Prior to the first use, add 1 ml **Resuspension Buffer** to a tube containing RNaseA lyophilizate and vortex. Transfer 1 ml of a solution RNaseA back into **Resuspension Buffer** and mix thoroughly. It is recommended to mark the bottle containing added RNaseA. Store at +4°C.

** Prior to the first use, add an appropriate amount of **96 – 100% ethanol to Wash Buffer** (see the instructions on the bottle label or in the table on the next page). It is recommended to mark the bottle containing added alcohol.

In order to avoid evaporation, ensure that the buffer bottles are tightly closed before storing.

Number of isolation	250 isolations
Wash Buffer	42 ml
96-100% ethanol	168 ml
Total volume	210 ml

Expiry date
Under proper storage conditions the kit will remain stable for at least 12 months from opening or until the expiry date.

III. | Additional materials and equipment required

· 96 – 100% ethanol PFA
· 1.5 – 2 ml sterile microcentrifuge tubes
· automatic pipettes and pipette tips
· disposable gloves
· microcentrifuge with rotor for 1.5 – 2 ml (≥ 11 000 x g)
· vortex mixer

IV. | Principle

DNA purification procedure utilizes spin minicolumns with membranes which efficiently and selectively bind nucleic acids. During the first step plasmid DNA is released from bacterial cells by alkaline lysis. Then lysate is neutralized and all cell residues along with proteins and genomic DNA are separated in centrifugation step. Lysate is applied to purification minicolumn membrane and DNA is bound. A two-step washing stage effectively removes impurities and enzyme inhibitors. Purified plasmid DNA is eluted with the use of a low ionic strength buffer (Elution Buffer) or water (pH 7.0 – 9.0) and may be used directly in all downstream applications such as PCR, qPCR, Southern blotting, DNA sequencing, enzymatic restriction, ligation and so forth, or stored until ready to use.

V. | Quality Control

The quality of each production batch (LOT) of the **extrAXON PLASMID MINI KIT** is tested using standard QC procedures. Purified DNA concentration and quality are evaluated by gel electrophoresis and spectrophotometer.

VI. | Product Specifications

SAMPLE MATERIAL

▶ Bacterial broth culture, frozen cell pellet – however the efficiency will be decreased

BINDING CAPACITY

Approx. 60 µg DNA

TIME REQUIRED

▶ Approx. 25 minutes

DNA PURITY

A_{260}/A_{280} ratio = 1.7 – 1.9

VII. | Safety Precautions

- Bacterial culture is treated as a biohazardous material of its potential pathogen content or health and life-threatening substances. While working with bacterial cultures, it is essential to comply with all safety requirements for working with biohazardous material.
- It is advisable to conduct the entire isolation procedure in a Class II Biological Safety Cabinet or at laboratory burner as well wearing disposable gloves and suitable lab coat at all times.
- The use of sterile filter tips is recommended.
- Avoid the cross contamination of DNA between minicolumns.
- Guanidine salts' residues may form highly reactive compounds when combined with oxidation components. In case of spillage, clean the surface with a detergent-water solution.
- In case of spillage of a liquid containing microorganisms, clean the contaminated surface with a detergent-water solution.

VIII. | Recommendations and important notes

SAMPLE MATERIAL

DNA isolation efficiency and purity can vary depending on a number of factors, such as plasmid copy number (high copy and low copy plasmids), cell density of bacterial culture, cell type (morphology), culture medium, growth phase, age and condition of cells. It is recommended to extract DNA from fresh, properly prepared starting material, which guarantees best isolation parameters. To minimize DNA degradation, avoid subjecting the sample material to repeated freeze/thaw cycles. Using old or repeatedly frozen/thawed material may result in low efficiency of isolation and poor DNA quality.

DNA ELUTION

Optimal volume of Elution Buffer used should be chosen in line with the quantity of sample material and final DNA concentration expected. Use of 50 – 100 µl Elution Buffer is recommended.

If a high DNA concentration is desired, elution volume may be reduced to 20 µl. It should be noted that this may reduce efficiency. It is essential to apply Elution Buffer precisely to the centre of the membrane.

In order to maximize DNA retrieval, heat Elution Buffer to 80°C and incubate it on membrane for 10 minutes.

If full DNA retrieval is required, 200 µl Elution Buffer should be used. However it will result in DNA dilution. Second elution can also be performed. For second elution, repeat steps 13 – 16 of the Isolation Protocol (section XI), placing purification minicolumn in a new, sterile 1.5 ml Eppendorf tube.

ELUTION BUFFER

Elution Buffer does not contain EDTA, which may interfere with some enzymatic reactions.

BALTICBLUE (OPTIONAL)

BalticBlue is an indicatior that provides visual identification of optimum buffer mixing. This prevents common handling errors that lead to inefficient cell lysis and incomplete precipitation of SDS, genomic DNA and cell debris.

BalticBlue should be added to Resuspension Buffer at a ratio of 1:1000 (e.g. 12.5 µl BalticBlue to 12.5 ml Resuspension Buffer). Mix thoroughly. BalticBlue precipitates. After addition of Lysis Buffer, precipitate dissolves and color changes to blue. During neutralization invert mixture gently until white precipitate is formed and blue solution will be colorless.

When isolating in parts, transfer enough of Resuspension Buffer for your isolations to a separate bottle/tube and add BalticBlue.

LOADING BUFFER

Loading Buffer is provided for analysis of purified DNA samples with the use of gel electrophoresis. Loading Buffer contains 3 dyes (bromophenol blue, xylene cyanol and orange G). Loading Buffer is concentrated by a factor of six, thus, in order to obtain the most satisfying results mix 2 µl of Loading Buffer with 10 µl of purified DNA.

IX. | Sample preparation

Bacterial culture preparation

It is essential to use fresh overnight broth culture in order to obtain high yield and no genomic DNA contaminations. Bacterial culture should be rejuvenated by streaking a single colony on a Luria-Bertani Agar (LA) plate containing suitable antibiotics. After incubation, select a single colony and use it to inoculate LB medium (containing antibiotic) and incubate overnight at 37°C with intense mixing (app. 200 rpm). Incubation should be terminated at a culture density of 1.0 – 6.0 A_{600} units per ml (12 – 16 hours). To provide optimal mixing and aeration conditions, use a flask which is at least four times larger than the culture volume. A baffled culture flask can also be used. In the table below gives information on concentrations of commonly used antibiotics.

Antibiotic	Recommended stock solution [mg/ml]	Solvent	Recommended working concentration [µg/ml]	Storage conditions
Ampicillin	100	H_2O	20 – 100	-20°C
Tetracycline	15	EtOH, 70%	10 – 50	-20°C
Chloramphenicol	34	EtOH, 70%	20 – 170	-20°C
Kanamycin	50	H_2O	10 – 50	-20°C
Streptomycin	50	H_2O	10 – 100	-20°C

In the table below shows the optimal culture volume that should be used for isolation according to the optical density at 600 nm.

OD_{600}	1	2	3	4	5	6
Culture volume (high copy plasmids)	15 ml	8 ml	**5 ml**	4 ml	3 ml	2 ml
Culture volume (low copy plasmids)	–	–	**10 ml**	8 ml	6 ml	4 ml

X. | Prior to isolation

1. Mix well each buffer supplied with kit. Mix **Lysis Buffer** very carefully.

2. Ensure that ethanol has been added to **Wash Buffer**. If it has not, add appropriate quantity of **96 – 100% ethanol**, volumes can be found on bottle labels or in table given in section II.

3. Ensure that RNase A has been added to **Resuspension Buffer** (section II).

4. Check if BalticBLue has been added to **Resuspension Buffer** (optional, section VIII).

5. Examine buffers. If a sediment has occurred in any of them, incubate it at **37°C (Lysis Buffer, Wash Buffer)** or at **50 – 60°C (Neutralization Buffer)** mixing occasionally until the sediment has dissolved. Cool to room temperature.

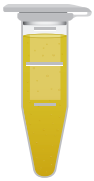
6. Unless otherwise stated, conduct all the isolation steps at room temperature.

XI. | Isolation Protocol

Ensure that ethanol has been added to Wash Buffer and that RNaseA has been added to Resuspension Buffer (section II). Check if BalticBlue has been added to Resuspension Buffer (optional).

3000 – 4000 x g

5 min



STEP 1

Pellet cell from bacterial culture by centrifugation for **5 min** at 3000 – 4000 x g.

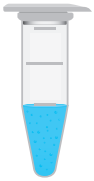
- For instructions, see section IX. Sample preparation.

STEP 2

a. Discard supernatant and resuspend cell pellet thoroughly in **250 µl Resuspension Buffer** by pipetting or vortexing.

- Incomplete resuspension may result in considerable decrease of yield.

Transfer resuspended cell pellet to a sterile 1.5 ml Eppendorf microcentrifuge tube.



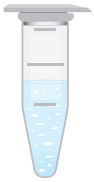
b. Add **250 µl Lysis Buffer** and mix carefully by inverting the tube 4 – 6 times.

- In order to avoid shearing of genomic DNA, sample has to be mixed gently. **Do not vortex!**

- After adding Lysis Buffer, sample should be clear. If not, incubate it at room temperature for 60 – 120 seconds. In order to avoid denaturation of supercoiled plasmid DNA, do not incubate longer than 5 minutes.
- If BalticBlue has been added to Resuspension Buffer, color changes to blue.

c. Add **350 µl Neutralization Buffer** and mix carefully by inverting tube several times.

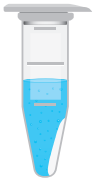
- Sample has to be mixed thoroughly and gently. **Do not vortex!**
- Cloudy solution and a white pellet is an effect of protein and genomic DNA precipitation.
- If BalticBlue has been added, suspension should be mixed until white precipitate has formed and blue has turned colorless without any traces of blue color.



STEP 3

Centrifuge for **5 min** at 11 000 x g.

- Repeat this step if supernatant is not clear.



STEP 4

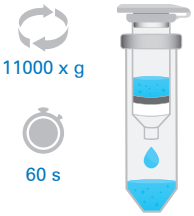
Carefully pipet supernatant containing plasmid DNA into a purification minicolumn placed in a collection tube. Centrifuge for **60 s** at 11 000 x g.

- Keep pipette tip away from pellet, which contains genomic DNA and cell remains.

Discard filtrate and reuse collection tube.

STEP 5

OPTIONAL STEP:
Add 500 µl **NW Buffer** and centrifuge for **60 s** at 11 000 x g. Discard filtrate and reuse collection tube.

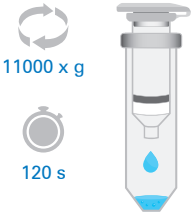


- This step is necessary if plasmid DNA is prepared from host strain containing high levels of nucleases.

Add **750 µl Wash Buffer** and centrifuge for **60 s** at 11 000 x g.

Discard filtrate and reuse collection tube.

STEP 6



Centrifuge for **120 s** at 11 000 x g.

- Wash buffer contains alcohol, which may interfere with some enzymatic reactions and also decrease elution efficiency. It is therefore vital to remove alcohol completely from minicolumn prior to the elution.

Discard collection tube and filtrate and carefully transfer purification minicolumn to a sterile 1.5 ml Eppendorf microcentrifuge tube.

STEP 7

Add **50 – 100 µl Elution Buffer**, directly onto purification minicolumn membrane.

- Other buffer volumes between 20 – 100 µl range may be used. For instructions, see section VIII. Recommendations and important notes.

Incubate minicolumn at room temperature for **60 s**.

Centrifuge at 11 000 x g for **60 s**.

Remove minicolumn. Isolated DNA should be stored at **+4°C or -20°C** depending on further applications.

XII. | Troubleshooting

Problem	Possible cause	Solution
Incomplete cell lysis.	Too many cells were taken for DNA purification.	Density of a bacterial culture should be equal to $A_{600} \leq 6.0$. For recommended sample volumes, see section IX. Sample preparation.
	Incomplete suspension of bacterial pellet in Resuspension Buffer.	Cell pellet should be mixed thoroughly in Resuspension Buffer by an intensive vortexing or pipetting until complete suspension.
	Salt precipitation in Lysis Buffer occurred.	When Lysis Buffer is stored below 20°C, a salt precipitation may occur. Redissolve any precipitate by warming solution at 37°C, then mix well and cool down to room temperature before use.
	Lysate is not clear.	Incubate lysate at room temperature for 60-120 seconds. Do not incubate for longer than 5 minutes to avoid denaturation of supercoiled plasmid DNA.
Plasmid DNA has denatured.	Prolonged incubation with Lysis Buffer.	Do not incubate sample for longer than 5 minutes before adding Neutralization Buffer.
Low yield of purified DNA.	Starting material contained few bacterial cells.	Increase amount of the starting material. For instructions, see section IX. Sample preparation.
	Old bacterial culture was taken for DNA isolation.	Culture cells in a broth medium containing antibiotic for no longer than 16 h.
	Bacterial cells do not contain plasmids.	Ensure that appropriate antibiotics were added to any culture medium used.
	Culture medium was not removed completely from cell pellet.	Carefully and accurately remove any residues of culture medium from above cell pellet.
	Incomplete cell lysis.	See «Incomplete cell lysis».
	Incomplete transfer of lysate into a purification minicolumn.	Clear supernatant may be poured directly into a purification column, however it is the most efficient to transfer lysate by a pipette.
	Ethanol was not added to Wash Buffer.	Ensure that 96 – 100% ethanol was added to Wash Buffer before use.
	pH of water used for elution is lower than 7.0.	Use Elution Buffer for DNA elution.

Problem	Possible cause	Solution
Low concentration of purified DNA.	Too much Elution Buffer was used.	Decrease volume of Elution Buffer. For details, see section VIII. Recommendations and important notes.
Isolated DNA is of poor purity.	Old bacterial culture has been processed.	Keep culture cells in broth medium containing antibiotic for no longer than 16 h.
	Culture medium was not removed completely from cell pellet.	Some medium components may affect DNA purity. LB medium is recommended for direct culture lysis. If another medium is used, pellet should be suspended in water or TE buffer prior to lysis. Make sure that a culture medium is completely removed from cell pellet
	One of washing steps was omitted.	Repeat isolation, performing both washing steps.
	Purified DNA contains residual alcohol.	Repeat the isolation paying a particular attention to whether any residual Wash Buffer is left in the purification minicolumn after centrifugation process during step 11.
Genomic DNA contamination present.	Old bacterial culture has been processed.	Keep culture cells in broth medium containing antibiotic for no longer than 16 h.
	Fragmentation of genomic DNA during cell lysis.	Do not vortex sample when Lysis Buffer has been added. It may cause genomic DNA fragmentation and contamination of purified plasmid DNA sample.
RNA contamination present.	RNaseA was not added to Resuspension Buffer.	Ensure that RNaseA was added to Resuspension Buffer before use.
	Improper storage of Resuspension Buffer.	Resuspension Buffer contains RNase A and must be stored at +4°C.
Inhibition of downstream enzymatic reactions.	Plasmid DNA has denatured.	See „Plasmid DNA has denatured".
	Purified DNA contains residual alcohol.	Repeat isolation, giving particular attention to ensuring that no residual Wash Buffer is left in purification minicolumn after centrifugation process during step 11.

XIII. | Safety information

extrAXON
PLASMID MINI KIT

Neutralization Buffer
H302, H315, H319
P264, P301+P312 P330

NW Buffer
H225, H315, H319, H336
P210, P304+P340 P312

Warning / Danger
H225 Highly flammable liquid and vapour. **H302** Harmful if swallowed. **H315** Causes skin irritation. **H319** Causes serious eye irritation. **H336** May cause drowsiness or dizziness. **P210** Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking. **P264** Wash hands thoroughly after handling. **P301+P312** IF SWALLOWED: Call a POISON CENTER/doctor if you feel unwell. **P330** Rinse mouth. **P304+P340** IF INHALED: Remove person to fresh air and keep comfortable for breathing. **P312** Call a POISON CENTER/ doctor if you feel unwell.

10910A 042018

Axon Lab AG
Täfernstrasse 15
CH-5405 Baden-Dättwil
info@axonlab.ch
www.axonlab.com

Axonlab
connecting ideas