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Research use only

Axonlab LifeScience
GENOMICS

extrAXON
PLASMID MIDI KIT

Kits for isolation of plasmid DNA in medium scale

The **extrAXON PLASMID MIDI KIT** is designed for the efficient purification of high quality plasmid DNA from 50-300 ml of cultured bacterial cells. The kit is based on anion-exchange resins, allowing extraction of ultrapure, transfection-grade pDNA, which is highly suited for use in demanding applications. The isolation protocol and buffer formulations were optimized for high isolation efficiency and pDNA purity. The product is intended for research use only.

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PLASMID MIDI KIT

Instructions to use
Version 062018

I. | Intended Use

The **extrAXON PLASMID MIDI KIT** is designed for the efficient purification of high quality plasmid DNA from 50-300 ml of cultured bacterial cells. The kit is based on anion-exchange resins, allowing extraction of ultrapure, transfection-grade pDNA, which is highly suited for use in demanding applications. The isolation protocol and buffer formulations were optimized for high isolation efficiency and pDNA purity. The product is intended for research use only.

II. | COMPONENTS OF THE KIT AND STORAGE CONDITIONS

Number of isolation	**25 isolations**
PMd1 Buffer* (Resuspension Buffer)	100 ml
PMd2 Buffer (Lysis Buffer)	100 ml
PMd3 Buffer (Neutralization Buffer)	100 ml
PMdQ Buffer (Equilibration Buffer)	125 ml
PMdW Buffer (Wash Buffer)	2 x 150 ml
PMdE Buffer (Elution Buffer)	200 ml
▶ RNase A (lyophilized)	1 pc
RNase A Buffer	1 ml
pDNA MIDI Purification Columns	25 pcs

* Before using for the first time, add the appropriate volume of **RNase A Buffer** to **RNase A lyophilizate** (see Table). Then, add prepared RNase A to the **PMd1 Buffer**. The PMd1 buffer with RNase A should be stored at +4°C for up to 6 months.

▶ **RNase A** is shipped lyophilized. It should be stored at +4°C. After reconstitution, the **RNase A** should be kept at +4°C for short-term storage (several days) or in aliquots at -20°C.

All the other components of the kit should be stored at room temperature (15 – 20°C). In order to avoid evaporation, ensure that the buffer bottles are tightly closed before storing. Under proper storage conditions, the kit will remain stable for at least 12 months.

III. | Additional materials and equipment required

· 50 ml centrifuge tubes
· automatic pipettes and pipette tips
· disposable gloves
· centrifuge with rotor for 50 ml tubes (≥3000 x g)
· dry block heater or water bath (up to 60°C)
· vortex mixer
· 75% ethanol, isopropanol, TE or ddH20

IV. | Principle

The plasmid DNA purification procedure utilizes pre-packed anion-exchange resin columns which efficiently and selectively bind nucleic acids. In the first isolation step, the pDNA is released from bacterial cells by alkaline lysis. Then the lysate is neutralized and all the cell residues along with the proteins and genomic DNA are separated in the centrifugation step. This alkaline lysis method and RNase treatment are used to obtain clear cell lysate with minimal genomic DNA/RNA contaminants. In the next step the lysate is applied to the purification column with the equilibrated resin and the DNA is bound. The washing stage effectively removes impurities and enzyme inhibitors. A suitable buffer with a high ionic strength allows the elution of the plasmid DNA, which is then concentrated and desalted by precipitation. The purified plasmid DNA may be used directly in all downstream applications such as PCR, qPCR, transfection, microinjection, Southern blotting, DNA sequencing, enzymatic restriction and so forth, or stored until ready to use.

V. | Quality Control

The quality of each production batch (LOT) of the **extrAXON PLASMID MIDI KIT** is tested with use of standard QC procedures. The purified DNA concentration, quality and stability are evaluated by gel electrophoresis and spectrophotometry. In addition, the functional quality is tested by qPCR, digestion with restriction enzymes and pDNA transfection.

VI. | Product Specifications

SAMPLE MATERIAL

▶ Bacterial broth culture:
50 – 150 ml (high-copy number plasmids)
100 – 300 ml (medium- and low-copy number plasmids)

YIELD

200 – 600 µg of transfection-grade pDNA from 100 ml of cultured bacterial cells

TIME REQUIRED

▶ 70 min
▶ additional ~60 min for DNA precipitation

DNA PURITY

A_{260}/A_{280} ratio = 1.7 – 1.9

VII. | Safety Precautions

· Bacterial culture is treated as a biohazardous material on account of its potential pathogen content or health and life-threatening substances. While working with bacterial cultures, all the safety requirements for working with biohazardous material is essential.
· Conducting the entire isolation procedure in a Class II Biological Safety Cabinet or at a laboratory burner is recommended, as is wearing disposable gloves and a suitable lab coat.
· The use of sterile pipette filter tips is recommended.
· 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

The DNA isolation efficiency and purity can vary depending on a number of factors, such as plasmid copy number (high copy and low copy plasmids), plasmid DNA size, 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. Moreover, the strain used to propagate a plasmid may have a substantial influence on the quality of the purified DNA. In some cases it is recommended to consider a change of a host strain. 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 the isolation and poor DNA quality.

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. A single colony may be previously use in order to inoculate the culture of 2 – 5 ml LB medium containing suitable selective antibiotics and incubate for approx. 8 h at 37°C with vigorous shaking (approx. 300 rpm). Then the starter culture should be diluted 1/500 to 1/1000 into a suitable volume of LB medium (50 – 300 ml) with selective antibiotics and incubate overnight (12 – 16 h) at 37°C with intense mixing (app. 200 rpm). Incubation should be terminated at a culture density of 2.0 – 5.0 A_{600} units per ml. 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. The optimal isolation parameters are obtained by processing 50 – 150 ml of bacterial culture (high-copy number plasmids) and 100 – 300 ml (medium- and low-copy number plasmids) for **extrAXON PLASMID MIDI KIT**.

Below you will find additional information to sample preparation which should ensure high isolation efficiency and pDNA purity.

		Recommended Culture Volume according to a culture density A_{600}		
Plasmid Type	**Pellet Wet Weight**	**OD_{600}=2**	**OD_{600}=4**	**OD_{600}=6**
High-copy number	0.75 g	150 ml	75 ml	50 ml
Low-copy number	1.12 g	300 ml	150 ml	100 ml

X. | Prior to isolation

1. Mix well each buffer supplied with the kit. Mix the **PMd2 Buffer** very carefully.

2. Examine the buffers. If a sediment has occurred in **PMd2 Buffer**, incubate it at 37°C. Cool to room temperature.

3. Pre-chill **PMd3 Buffer** at 4°C.

XI. | Isolation Protocol

1. Pellet cells from 50 – 300* ml of bacterial culture by centrifugation for 15 min at 6000 x g.

* The optimal isolation parameters are obtained by processing 50-150 ml of bacterial culture (high-copy number plasmids) and 100 – 300 ml (medium- and low-copy number plasmids).

   - In order to obtain a cell pellet from >50 ml of bacterial culture, after the centrifugation 50 ml of culture, discard the supernatant and repeat this step using the same 50 ml centrifuge tube.

2. During step one, equilibrate the pDNA MIDI Purification Column. Place the column in a new 50 ml collection tube (not provided) and add **5 ml PMdQ Buffer**. Allow the column to empty by gravity flow.
   The column is ready for step 7.

3. Discard the supernatant completely, and resuspend the cell pellet thoroughly in **4 ml PMd1 Buffer** by pipetting or vortexing.
   - Incomplete resuspension may result in considerable decrease of yield.
   - Incomplete removal of supernatant may affect the purity of isolated plasmid DNA.

4. Add **4 ml PMd2 Buffer** and mix carefully by inverting the tube (approx. 10 times).
   - In order to avoid shearing of the genomic DNA, the sample has to be mixed gently. **Do not vortex!**
   - After adding the PMd2 Buffer, sample should be clear. If not, incubate it at room temp. for 3 min. In order to avoid denaturation of the supercoiled plasmid DNA, do not incubate longer than 5 min.

5. Add **4 ml pre-chilled PMd3 Buffer** and mix carefully by inverting the tube several times (approx. 10 times). Incubate the lysate for 2 – 5 min.
   - It is recommended to used chilled PMd3 Buffer in order to enhance the precipitation.

- The 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.

6. Centrifuge for 20 min at ≥6000 x g. If supernatant is not clear, centrifuge for another 20 min at ≥6000 x g.

7. Carefully pipet the supernatant containing the plasmid DNA into the equilibrated pDNA MIDI Purification Column placed in a 50 ml collection tube (prepared during step 2). Allow the column to empty by gravity flow.
   - Keep the pipette tip away from the pellet, which contains genomic DNA and cell remains.

8. Add **12 ml PMdW Buffer.** Allow the column to empty by gravity flow.

9. Place the column in a new 50 ml centrifuge tube. Eluate DNA with **8 ml PMdE Buffer** by gravity flow.

10. Precipitate DNA by adding **6 ml of isopropanol** (not provided). Mix the tube and centrifuge at ≥6000 x g (preferably at 15000 x g) for 30 min at 4°C.

11. Carefully remove the supernatant. Wash the pellet with **5 ml of 75% ethanol** (not provided). Centrifuge at ≥6000 x g (preferably at 15000 x g) for 10 min. Carefully remove the supernatant. Air-dry pellet for approx. 10 min and add a suitable volume of TE buffer or DNase-free water.
    - To dissolve the DNA pellet the tube may be placed in a 60°C dry block heater or water bath for 10 min.

XII. | Troubleshooting

Problem	Possible cause	Solution
Incomplete cell lysis.	Too many cells were taken for DNA purification.	The bacterial culture should be at a density of $A_{600} \leq 5,0$. For recommended sample volumes, see section IX. Sample preparation.
	Incomplete suspension of the bacterial pellet in the PMd1 Buffer.	The cell pellet should be mixed thoroughly in the PMd1 Buffer by intensive vortexing or pipetting until complete suspension.
	Salt precipitation in PMd2 Buffer occurred.	When PMd2 Buffer is stored below +20°C, a salt precipitation may occur. Re-dissolve any precipitate by warming the solution at 37°C, then mix well and cool down to the room temperature before use.
	The lysate is not clear.	Incubate the lysate at room temperature for 3 min. Do not incubate for longer than 5 min to avoid denaturation of supercoiled plasmid DNA.
Low yield of purified DNA.	Starting material contained low amount of bacterial cells.	Increase the 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.
	The bacterial cells do not contain plasmids.	Ensure that the appropriate antibiotics were added to every culture medium used.
	The culture medium was not removed completely from the cell pellet.	Carefully and accurately remove any residues of culture medium from above cell pellet.
	Incomplete cell lysis.	See the problem: «Incomplete cell lysis».
	Incomplete transfer of the lysate into a purification column.	Transfer the lysate into the pDNA MIDI Purification Column by a pipette.
	The purification column was not equilibrated.	Ensure that the pDNA MIDI Purification Column was equilibrated with the PMdQ Buffer.

Problem	Possible cause	Solution
Plasmid DNA has denatured.	Prolonged incubation with the PMd2 Buffer.	Do not incubate the sample for longer than 5 min before adding the PMd3 Buffer.
Isolated DNA is of poor purity.	Old bacterial culture has been processed.	Culture cells in broth medium containing antibiotic for no longer than 16 h.
	The culture medium was not removed completely from the cell pellet.	Some medium components may affect DNA purity. The LB medium is recommended for direct culture lysis. If another medium is used, the pellet should be suspended in water or TE buffer prior to lysis. Ensure complete removal of the culture medium from over the pellet.
Genomic DNA contamination present.	Old bacterial culture has been processed.	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 the PMd2 Buffer has been added. It may cause the genomic DNA fragmentation and contamination of purified plasmid DNA sample.
RNA contamination present.	Improper preparation of PMd1 Buffer.	Add RNase A to the PMd1 Buffer.
	Improper storage of PMd1 Buffer.	The PMd1 Buffer with RNase A must be stored at +4°C.
Inhibition of downstream enzymatic reactions.	The plasmid DNA has denatured.	See the problem: «Plasmid DNA has denatured».

XIII. | Safety information

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PMdQ Buffer
H225, H319
P264, P305+P351+P388, P210

PMdW Buffer
H225, H319
P264, P305+P351+P388, P210

PMdE Buffer
H225, H319
P264, P305+P351+P388, P210

Danger
H225 Highly flammable liquid and vapour. **H319** Causes serious eye irritation. **P264** Wash hands thoroughly after handling. **P305+P351+P388** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. **P210** Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

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