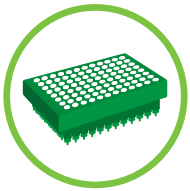
EXTRACTME®
PLASMID DNA 96-WELL KIT

Cat. no. EM21
Version: 1.2017

Plasmid DNA 96-Well Kit



I. INTENDED USE

The ***EXTRACTME*** **PLASMID DNA 96-WELL KIT** is designed for high-throughput 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 ISOLATIONS	2x96 WELL PLATES/KIT	10x96 WELL PLATES/KIT
Catalogue number	EM21-192	EM21-960
⚠ **Resuspension Buffer**	48 ml	240 ml
Lysis Buffer	48 ml	240 ml
Neutralization Buffer	67.5 ml	337.5 ml
Wash Buffer (conc.)*	56 ml	5 x 56 ml
Elution Buffer	38.5 ml	5 x 38.5 ml
Plasmid collection Plates	2 pcs	10 pcs
Plasmid Binding Plates	2 pcs	10 pcs
Plasmid Elution Plates	2 pcs	10 pcs
Elution Adhesive Seals	2 pcs	10 pcs

*Before using for the first time, add the appropriate quantity of 96-100% ethanol to the **Wash Buffer**; for details, see the instructions on the bottle label and in the table below. Mark the bottle after adding the alcohol is recommended.

⚠ **Resuspension Buffer should be stored at +4°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.

NUMBER OF ISOLATIONS	2x96 WELL PLATES/KIT	10x96 WELL PLATES/KIT
Catalogue number	EM21-192	EM21-960
Wash Buffer	56 ml	5 x 56 ml
96-100% ethanol	224 ml	5 x 224 ml
Total volume	280 ml	5 x 280ml

III. ADDITIONAL MATERIALS AND EQUIPMENT REQUIRED

- → 96-100% ethanol PFA
- → automatic pipettes and pipette tips
- → disposable gloves
- → microcentrifuge with rotor for plates (>3k x g)
- → dry block heater or water bath (up to 70°C)
- → vortex
- → 96 Deep Well Plates for bacterial culture and samples preparation
- → adhesive film

IV. PRINCIPLE

The DNA purification procedure utilizes spin 96-minicolumns plates with membranes which efficiently and selectively bind nucleic acids. In the first isolation step, the plasmid DNA 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. The lysate is applied to the purification minicolumn membrane and the DNA is bound. The two-step washing stage effectively removes impurities and enzyme inhibitors. The purified plasmid DNA is eluted using 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 ***EXTRACTME*** **PLASMID DNA 96-WELL KIT** is tested using s tandard QC procedures. The purified DNA concentration, quality and stability are evaluated by gel electrophoresis and spectrophotometer.

VI. PRODUCT SPECIFICATIONS

SAMPLE MATERIAL
Bacterial broth culture
BINDING CAPACITY
Approx. 25 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 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.
- → Avoid the cross-transferral of DNA between minicolumns.
- → Guanidine salts residues may form highly reactive compounds when combined with oxidation compounds. 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

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), 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 the isolation and poor DNA quality.

DNA elution

The optimal volume of the elution buffer used should be chosen in line with the quantity of the sample material and the final DNA concentration expected. The use of 50-100 µl Elution Buffer is recommended.

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

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

If full DNA retrieval is required, 200 µl or more Elution Buffer should be used. However it will result in the DNA dilution. Second elution can also be performed. For the second elution, repeat steps 14-17 of the Isolation Protocol (section XI), placing Plamid binding plate in a new Elution Plate (optional not included in the kit).

Elution Buffer

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

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 the 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 2.0-5.0 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.

The optimal isolation parameters are obtained by processing 1.5-3 ml of culture. In some cases (for example, low density of bacterial cultures, medium and low copy number plasmids), it may be necessary to use larger volumes of bacterial culture.

Bacterial Cultures in a 96 deep-well plate

Transfer 1.3 ml of fresh LB medium containing appropriate antibiotics into each well of a 96 Deep Well Plate then inoculate each well with a single bacterial colony. Seal the plate with microporous tape or Adhesive Film. When using non-porous adhesive tape, pierce 2-3 holes in the tape with a needle above each well to promote air exchange. Incubate at 37°C for 20-24 hours with 180-250 rpm shaking. Pellet the bacterial culture in the plate by centrifugation for 5 min at 2,100 x g. During centrifugation, the plate should be covered with Adhesive Film. Following centrifugation, remove the Adhesive Film then remove the supernatant in each well by quickly inverting the plate.

Isolation from high copy number plasmids

(such as pUC, pBluescript®, pGEM®, pJET, pTZ and derivatives)
For DNA isolation from high copy number plasmids, process pellets from 0.5-5 ml of bacterial culture. Do not exceed 5 ml, as this may result in clogging of the purification minicolumn and no increase in DNA yield will be obtained.

Isolation from medium and low copy number plasmids
(such as pBR322, pET, pACYC, pSC101 and derivatives, cosmids)
For DNA isolation from medium and low copy number plasmids, it may be necessary to increase the sample volume by as much as up to 10 ml in order to recover a sufficient quantity of DNA. The volumes of the buffers used for isolation should not be altered. N.B.: increasing the sample volume to over 10 ml may result in clogging of the purification minicolumn and a decreased DNA yield.

X. BEFORE STARTING

1. Mix well each buffer supplied with the kit. Mix the **Lysis Buffer** very carefully.
2. Ensure that ethanol has been added to the **Wash Buffer**. If it has not, add appropriate quantity of 96-100% ethanol, the volumes can be found on the bottle labels or in the table given in section II.
3. Examine the 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.
4. Heat a sufficient quantity of the **Elution Buffer** to 70°C.
5. Unless otherwise stated, conduct all the isolation steps at room temperature.

XI. ISOLATION PROTOCOL

1. Add **250 µl of Resuspension Buffer** to each well of the 96 Deep Well Plate or microcentrifuge tubes then resuspend the cell pellet by pipette. Continue to pipette until all traces of the cell pellet have been dissolved.
   - ⚠ Transfer the resuspended cell samples from microcentrifuge tubes to each well of a new 96 Deep Well Plate.
   - ⚠ Incomplete resuspension may result in considerable decrease of yield.
2. Add **250 µl Lysis Buffer** to each well of the 96 Deep Well Plate. Dry the top of the plate with paper towel then seal tightly with new Adhesive Film. Gently invert the plate 6-8 times.
   - ⚠ In order to avoid shearing of the genomic DNA, the sample has to be mixed gently. **Do not vortex!**
   - ⚠ After adding the Lysis Buffer, sample should be clear. If not, incubate it at room temperature for 1-2 min. In order to avoid denaturation of the supercoiled plasmid DNA, do not incubate longer than 5 min.
3. Remove the Adhesive Film from the 96 Deep Well Plate then add **350 µl Neutralization Buffer** to each well. Dry the top of the plate with paper towel then seal tightly with new Adhesive Film. Gently invert the plate 6-8 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.
4. Centrifuge the 96 Deep Well Plate at 3000 x g for 10 min.
5. Place the Plasmid Binding Plate on a Plasmid Collection Plate.
6. Remove the Adhesive Film from the 96 Deep Well Plate then carefully pipet the supernatant containing the plasmid DNA to each well of the Plasmid Binding Plate.
   - ⚠ Keep the pipette tip away from the pellet, which contains the genomic DNA and cell remains.

7. Centrifuge the Plasmid Binding Plate with Plasmid Collection Plate at 3000 x g for 5 min.
8. Discard the filtrate and reuse the Plasmid Collection Plate.
9. Add **600 µl Wash Buffer** and centrifuge for 30 s at 3000 x g.
10. Discard the filtrate and reuse the Plasmid Collection Plate.
11. Repeat steps 9-10.
12. Centrifuge for 1-2 min at 3000 x g.

    ⚠ The Wash Buffer contains alcohol, which may interfere with some enzymatic reactions and also decrease the elution efficiency. It is therefore vital to remove the alcohol completely from the minicolumn before elution.

13. Discard the Plasmid Collection Plate and the flow-through and carefully transfer the Plasmid Binding Plate to a sterile Plasmid Elution Plate.
14. Add **50-100 µl Elution Buffer**, pre-heated to 70°C directly onto the purification minicolumn membrane.

    ⚠ Other buffer volumes in the 20-200 µl range may be used. For instructions, see section VIII. Recommendations and important notes.

15. Incubate the Plasmid Elution Plate at room temperature for 2 min.
16. Centrifuge at 3000 x g for 1 min.
17. Remove the Plasmid Binding Plate then seal tightly Plasmid Elution Plate with Elution Adhesive Seal. The isolated DNA is ready for use in downstream applications or for short-term storage at +4°C or for long-term storage at -20°C.

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 Resuspension Buffer.	The cell pellet should be mixed thoroughly in the 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 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 1-2 min. Do not incubate for longer than 5 min to avoid denaturation of supercoiled plasmid DNA.
Plasmid DNA has denatured	Prolonged incubation with the Lysis Buffer.	Do not incubate the sample for longer than 5 minutes before adding the Neutralization Buffer.
Low yield of purified DNA	Starting material contained few 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 any culture medium used.
	The culture medium was not removed completely from the cell pellet.	Carefully and accurately remove any residues of the culture medium from above the cell pellet.
	Incomplete cell lysis.	See „Incomplete cell lysis".
	Incomplete transfer of the lysate into a purification minicolumn.	The clear supernatant can be poured directly into a purification column, however it is the most efficient to transfer the lysate by a pipette.
	Ethanol was not added to the Wash Buffer.	Ensure that 96-100% ethanol was added to the Wash Buffer before use.
	The pH of the 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 the volume of the Elution Buffer. For details, see section VIII. Recommendations and important notes.
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.
	One of the washing steps was omitted.	Repeat the isolation, performing both washing steps.
	The purified DNA contains residual alcohol.	Repeat the isolation, giving particular attention to ensuring that no residual Wash Buffer is left in the purification minicolumn after centrifugation in step 12.
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 Lysis Buffer has been added. It may cause the genomic DNA fragmentation and contamination of purified plasmid DNA sample.
RNA contamination present	Improper storage of Resuspension Buffer.	The Resuspension Buffer contains RNase A and must be stored at +4°C.
Inhibition of downstream enzymatic reactions	The plasmid DNA has denatured.	See „Plasmid DNA has denatured".
	The purified DNA contains residual alcohol.	Repeat the isolation, giving particular attention to ensuring that no residual Wash Buffer is left in the purification minicolumn after centrifugation in step 12.

XIII. SAFETY INFORMATION

Neutralization Buffer	
	**Caution** H302, H315, H319 P305+P351+P338, P302+P352
Lysis Buffer	
	**Hazard** H315, H319 P280, P305+P351+P338

H302 Harmful if swallowed. **H315** Causes skin irritation. **H319** Causes serious eye irritation. **P280** Wear protective gloves/protective clothing/eye protection/face protection. **P305 + P351 + P338 IF IN EYES:** Rinse continuously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing. **P302+P352 IF ON SKIN:** Wash with plenty of soap and water.

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