MEBEP TECH(HK) Co., Limited
Email: sales@mebep.com Website: www.mebep.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

EndoFree Plasmid Mini Kit

Product Number: PLK0402

Shipping and Storage

1. When using for the first time, add all RNase A carried by the reagent kit to Buffer P1 (final concentration 100ug/ml) and store at 2-8℃. If RNase A is inactivated in Buffer P1, there may be trace RNA residues in the extracted plasmid. Adding RNase A to Buffer P1 is sufficient.
2. When the ambient temperature is low, SDS in Buffer P2 may precipitate turbidity or sediment. It can be heated in a 37℃ water bath for a few minutes to restore clarity. Do not shake violently to avoid excessive foam formation.
3. To avoid volatilization, oxidation, and pH changes caused by prolonged exposure of reagents to the air, each solution should be covered tightly in a timely manner after use.
4. Buffer ER can be transported at room temperature and stored at 4 ℃ for one month. It can be stored for a long time at -20 ℃.

Components

Component	Storage	PLK0402 50 Preps
Balance Buffer	RT	5ml
RNaseA(10mg/ml)	-20℃	150μl
Buffer P1	4℃	15 ml
Buffer P2	RT	15 ml
Buffer N3	RT	15 ml
Buffer PE	RT	16 ml
Buffer ER	-20℃	5ml
Buffer WB	RT	15 ml
Buffer EB	RT	15ml
Adsorption column AC	RT	50
Collection tube (2ml)	RT	50

Description

This reagent kit uses an improved SDS alkaline lysis method to lyse cells. The unique Buffer ER selectively binds to centrifugation to remove endotoxins. Then, the silica matrix membrane in the centrifugation adsorption column selectively binds to plasmid DNA in the solution under high salt and low pH conditions. Impurities and other bacterial components are removed through Buffer PE and Buffer WB. Finally, the pure plasmid DNA is washed off the silica matrix membrane with low salt and high pH Buffer EB.

Features

1. The silicon matrix membrane inside the centrifugal adsorption column is entirely made of specially designed adsorption membranes from world-renowned imported companies, with minimal differences in adsorption capacity between columns and good repeatability. Overcoming the drawback of unstable membrane quality in domestic reagent kits.
2. The unique process formula eliminates endotoxins, with extremely low endotoxin content (<0.1EU/μg DNA) and excellent cell transfection effect. It can also be directly used for various molecular biology experiments such as enzyme digestion, transformation, PCR, in vitro transcription, sequencing, etc.

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to

13000rpm.

2. The amount of plasmid extracted is related to factors such as bacterial culture concentration and plasmid copy number. For general high copy plasmids, it is recommended to inoculate a single colony in 1.5-4.5 ml LB medium with appropriate antibiotics, and incubate overnight for 14-16 hours to extract up to 20μg-50μg of pure plasmids. If the extracted plasmid is a low copy plasmid or a large plasmid larger than 10kb, the amount of bacterial cells used should be appropriately increased. 5-10 ml of overnight culture should be used, and the amount of P1, P2, and N3 should be increased proportionally. The other steps are the same.
3. The concentration and purity of the obtained plasmid DNA can be detected by agarose gel electrophoresis and ultraviolet spectrophotometer. A OD260 value of 1 is equivalent to approximately 50μg/ml of DNA. Electrophoresis may consist of a single band, as well as 2 or more DNA bands, mainly due to varying degrees of superhelix conformational plasmids swimming in different positions, which are related to the length of extract culture time and the intensity of extraction operations. Under normal operating conditions, our company's products can basically exceed 90% of the super spiral.
4. The exact molecular size of plasmid DNA can only be determined by comparing DNA molecular weight markers after enzyme tangent normalization. Plasmids in a circular or super spiral state have an uncertain swimming position and their exact size cannot be determined by electrophoresis.
5. Buffer EB does not contain chelating agent EDTA and does not affect downstream enzyme cleavage, linking, and other reactions. Water can also be used for elution, but it should be ensured that the pH is greater than 7.5, as low pH can affect elution efficiency. Plasmids washed with water should be stored at -20 ℃. If plasmid DNA needs to be stored for a long time, it can be eluted with TE buffer (10mM Tris HCl, 1mM EDTA, pH 8.0), but EDTA may affect downstream enzyme digestion reactions and can be diluted appropriately when used.

Use of balance buffer

1. Description

During the long-term placement of nucleic acid adsorption silica gel membrane columns, they react with charges/dust in the air and affect their nucleic acid binding ability.After pre-treatment with equilibrium solution, the silica gel column can greatly reduce the hydrophobic groups of the silica gel membrane in the column and improve the binding ability of nucleic acids.Thus improving the recovery efficiency or yield of silicone columns. The equilibrium solution is a strong alkaline solution. If accidentally touched, please clean it with a large amount of tap water.After use, the bottle cap needs to be tightly closed to avoid contact with air. Store at room temperature. During storage, there may be precipitation formation. Please heat to 37℃ to completely eliminate the precipitation.

2. Protocol

Take a new silicone membrane adsorption column and place it in a collection tube. Take 100μl of Balance Buffer and transfer it into the column. Centrifuge at 13000rpm for 1 minute, discard the waste liquid in the collection tube, and place the adsorption column back into the collection tube. At this point, the Balance Buffer has completed preprocessing the columns. Follow the subsequent operating steps.

Protocol(Please read the precautions before the experiment)

Note:1)Before the first use, please add the specified amount of anhydrous ethanol to the Buffer WB bottle and mix thoroughly. After addition, please mark the box with a check mark indicating that ethanol has been added in a timely manner to avoid multiple additions!

2)Add all RNase A to Buffer P1, mix well, and store at 2-8 ℃ after each use.

1. Take 1.5-4.5 ml of overnight cultured bacterial solution, centrifuge at 12000 rpm for 30 seconds, pour out the supernatant as much as possible, and collect the bacterial cells.

   Collect more than 1.5ml of bacterial solution. After centrifuging and discarding the supernatant, add more bacterial solution to the same 1.5ml tube and repeat step 1 until sufficient bacterial cells are collected.
2. Resuspend bacterial precipitation with 250μl Buffer P1 and vortex oscillate until completely suspended.

   If there are incompletely mixed bacterial blocks, it will affect the lysis, resulting in low extraction amount and purity.

3. Add 250μl of Buffer P2 and gently flip up and down 6-8 times to fully lyse the bacterial cells. Leave at room temperature for 4 minutes.

   Mix gently and do not shake vigorously to avoid genomic DNA cleavage! The time used should not exceed 5 minutes! To prevent plasmid damage. At this point, the bacterial liquid should become clear and viscous. If there are few bacterial bodies, the next step can be taken quickly after the clear and viscous consistency is achieved, not necessarily accurate for 5 minutes.

4. Add 250μl Buffer N3 and gently flip up and down 6-8 times immediately. When thoroughly mixed, white flocculent precipitates will appear. Centrifuge at 13000rpm for 10 minutes, carefully transfer the supernatant to a new tube to avoid absorbing floating white sediment.

   After adding Buffer N3, it should be mixed immediately to avoid local precipitation of SDS.

5. Add 0.1 volume (10% of the volume of supernatant, approximately 80μl) of buffer ER to the supernatant obtained in the previous step, invert and rotate until well mixed. Place in an ice bath or insert into crushed ice (or freezer) for 5 minutes until it becomes clear and transparent (or still slightly cloudy), occasionally mixing a few times in between.

   After adding buffer ER to the supernatant, it will become cloudy, but it should return to a clear (or slightly cloudy) state after ice bath.

6. Leave at room temperature for 3-5 minutes, and when the temperature returns to room temperature, the solution quickly turns turbid. Invert and mix well.

   If the indoor temperature is low or you want to speed up, you can take a water bath at 37-42 ℃, which will quickly become turbid and mix upside down.

7. Centrifuge at room temperature of 14000×g for 10 minutes to separate phases. The upper aqueous phase contains DNA, while the lower blue oily phase contains endotoxins and other impurities. Transfer the upper aqueous phase containing DNA to a new tube (be careful not to inhale the blue oily layer, which contains impurities such as endotoxins), and discard the oily layer.

   Balance Buffer pre-treatment adsorption column: The use of Balance Buffer pre-treatment of silicone membrane adsorption column is a necessary step. For specific methods, please refer to the previous section "About the Use of Balance Buffer"

8. Add 0.5 volume of isopropanol (about 370μl) to the upper aqueous phase, thoroughly invert and mix well, then divide into two parts (each time not exceeding 700μl) and transfer to the adsorption column AC (the adsorption column is placed in the collection tube). Centrifuge 12000×g for 1 minute and discard the waste liquid in the collection tube. Until all mixed solutions pass through this adsorption column.

9. Add 500μl of Buffer PE, centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.

   This step is to remove trace amounts of nucleases and other impurities. If the strains used are JM series, HB101 and other endA strains or wild-type strains with abundant nuclease content, this step should be added; If the strains used are XL-1 Blue, Top10, and DH5 α If the nucleic acid enzyme content is low in the defective strain, this step can be skipped.

10. Add 600μl Buffer WB (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid. Add 600μl Buffer WB and rinse again.

11. Put the adsorption column AC back into the empty collection tube, centrifuge at 12000 rpm for 2 minutes, and try to remove Buffer WB as much as possible to avoid residual ethanol in Buffer WB inhibiting downstream reactions.

12. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 50-100μl of Buffer EB to the middle of the adsorption membrane (Buffer EB is better heated in a 65-70 ℃ water bath beforehand). Leave at room temperature for 2 minutes and centrifuge at 12000 rpm for 1 minute. If a large amount of plasmid is required, the obtained solution can be re added to the centrifuge adsorption column, left at room temperature for 2 minutes, and centrifuged for 1 minute.

    The larger the elution volume, the higher the elution efficiency. If a high plasmid concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 30μl. If the volume is too small, it will reduce the efficiency of plasmid elution and reduce plasmid production.