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

High Pure Plasmid Mid Scale Mini Kit

Product Number: PLK1801

Shipping and Storage

1. When using for the first time, add all RNaseA carried by the reagent kit to Buffer P1 (final concentration 100μg/ml) and store at 2-8℃. If RNaseA is inactivated in Buffer P1, there may be trace RNA residues in the extracted plasmid. Adding RNaseA 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.

Components

Component	Storage	PLK1801 50 Preps
Balance Buffer	RT	5ml
RNaseA （10mg/ml）	-20℃	250μl
Buffer P1	4℃	25ml
Buffer P2	RT	25ml
Buffer N3	RT	25ml
Buffer PE	RT	16ml
Buffer WB	RT	15ml
Buffer EB	RT	15ml
Adsorption column AC	RT	50
Collection tube (2ml)	RT	50

Description

This kit uses a unique high-yield SDS alkaline lysis formula to lyse cells, resulting in a 1-2 fold increase in plasmid production. The silicon matrix membrane in the centrifugal adsorption column selectively binds plasmid DNA in the solution under high salt and low pH conditions, and then removes impurities and other bacterial components through Buffer PE and Buffer WB. Finally, the pure plasmid DNA is eluted from the silicon matrix membrane using low salt and high pH Buffer EB.

Features

1. The specially improved high-yield buffer formula can increase plasmid production by 1-2 times.
2. The unique Buffer PE formula can efficiently remove residual nucleases, even strains with abundant nuclease content such as JM series and HB101 can be easily removed. Effectively preventing plasmid degradation by nucleases.
3. Fast and convenient, without the need for toxic reagents such as phenol and chloroform, and without the need for ethanol precipitation. The obtained plasmids have high yield and good purity, and can be directly used for various molecular biology experiments such as enzyme digestion, transformation, PCR, in vitro transcription, sequencing, etc.

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.

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to 13000rpm, such as Eppendorf 5415C or a similar centrifuge.
2. The amount of plasmid extracted is related to factors such as bacterial culture concentration and plasmid copy number. Generally, high copy plasmids can be cultured overnight for 14-16 hours, and 5-15ml of culture can extract up to 40-90μg 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, 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). However, EDTA may affect downstream enzyme digestion reactions and can be diluted appropriately when used.

Protocol(Please read the precautions before the experiment)

Tip: 1) Before the first use, please add the specified amount of anhydrous ethanol to the Buffer WB bottle and Buffer PE bottle, mix well, and mark the added ethanol in the box in a timely manner to avoid multiple additions!

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

1. Take 5-15 ml of overnight cultured bacterial solution, centrifuge at 9000rpm for 1-2 minutes, pour out the supernatant as much as possible, and collect the bacterial cells.
2. Resuspend the bacterial precipitate with 500μl Buffer P1, vortex oscillate to complete suspension, and transfer all into a 2ml centrifuge tube.

   If there are incompletely mixed bacterial blocks, it will affect the lysis, resulting in low extraction amount and purity.
3. Add 500μl of Buffer P2, gently flip up and down 6-8 times to fully lyse the bacterial cells, and 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 500µl Buffer N3 and gently flip it up and down 6-8 times. 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.

   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"

5. Add 0.5 volume of isopropanol (about 740µl) to the upper aqueous phase, thoroughly invert and mix, then divide into several 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.
6. Add 500µl Buffer PE (please check if anhydrous ethanol has been added first!), centrifuge at 12000rpm for 30 seconds, and discard the waste liquid.

   In order to remove trace impurities such as nucleases, this step should be added if the strains used are JM series, HB101 and other endA strains or wild-type strains with abundant nuclease content; If the strains used are defective strains such as XL-1Blue, Top10, and DH5 with low nuclease content, this step can be skipped.

7. 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.
8. 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.
9. Take out the adsorption column AC and place it in a clean centrifuge tube. Add 100-200µl Buffer EB to the middle of the adsorption membrane (Buffer EB is better heated in a water bath at 65-70℃ 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 100µl. If the volume is too small, it will reduce the efficiency of plasmid elution and reduce plasmid production.