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 Mid Scale Mini Kit

Product Number: PLK1001

Shipping and Storage

1. when used for the first time, add all RNase A carried by the kit to buffer P1 (final concentration 100μg/ml) were stored at 2-8℃. If RNase A is inactivated in buffer P1, the extracted plasmid may have trace RNA residue. Add RNase A to buffer P1.
2. when the ambient temperature is low, SDS in buffer P2 may precipitate turbidity or precipitation. 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. avoid volatilization, oxidation and pH change of reagents exposed to air for a long time, and close the cover of each solution in time after use.
4. Buffer Er is transported at room temperature. It can be stored at 4℃ for one month, and stored at -20℃ for a long time.

Components

Component	Storage	PLK1001 50 Preps
Balance Buffer	RT	5ml
RNaseA(10mg/ml)	-20℃	250μl
Buffer P1	4℃	25 ml
Buffer P2	RT	25 ml
Buffer N3	RT	25 ml
Buffer PE	RT	16 ml
Buffer ER	-20℃	10 ml
Buffer WB	RT	15 ml
Buffer EB	RT	15ml
Adsorption column AC	RT	50
Collection tube (2ml)	RT	50

Description

This kit uses an improved SDS alkaline lysis method to lyse cells. Endotoxin is removed by a unique buffer Er selective combination with centrifugation. Then the silicon matrix membrane in the centrifugal adsorption column selectively binds plasmid DNA in the solution under high salt and low pH conditions. Impurities and other bacterial components are removed by buffer PE and buffer WB. Finally, the pure plasmid DNA is eluted from the silicon matrix membrane by buffer EB with low salt and high pH.

Features

1. The silicon matrix membranes in the centrifugal adsorption column are all specially made adsorption membranes imported from world-famous companies, with minimal difference in adsorption capacity between columns and good repeatability. It overcomes the disadvantage of unstable quality of domestic kit membrane.
2. The unique process formula can remove endotoxin, and the endotoxin content is very low (<0.1eu/μg DNA), and the cell transfection effect was excellent. It can also be directly used in enzyme digestion, transformation, PCR, in vitro transcription, sequencing, and other molecular biology experiments.

Note

1. All centrifugation steps are completed at room temperature, using a traditional table centrifuge with a rotational speed of 13000rpm, such as Eppendorf 5415c or similar centrifuges.
2. The amount of plasmid extracted is related to factors such as bacterial culture concentration and plasmid copy number.

Generally, high copy plasmids are cultured overnight for 14-16 hours, and up to 30-90μg of pure plasmids can be extracted from 5-15ml of culture. If the plasmid is a low copy plasmid or a large plasmid larger than 10KB, the amount of bacteria should be appropriately increased, and the amount of P1, P2, N3 should be increased proportionally. Other steps are the same.

3. The concentration and purity of the obtained plasmid DNA can be detected by agarose gel electrophoresis and UV spectrophotometer. An od260 value of 1 corresponds to approximately 50μg/ml DNA. The electrophoresis may be a single band or two or more DNA bands, which is mainly caused by the different swimming positions of the supercoiled conformation plasmids in different degrees, and is related to the length of culture time of the extract and the intensity of operation during extraction. The company's products can basically exceed 90% supercoiled under normal operation.
4. The exact molecular size of plasmid DNA can only be known by comparing the DNA molecular weight marker after enzymatic tangent characterization. The swimming position of plasmids in circular or supercoiled state is uncertain, and its exact size cannot be known by electrophoresis.
5. Buffer EB does not contain chelator EDTA and does not affect downstream enzyme digestion, ligation and other reactions. Water elution can also be used, but it should be ensured that the pH is greater than 7.5, and too low pH affects the elution efficiency. Plasmids eluted with water should be stored at -20°C. 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 the downstream enzyme digestion reaction, which can be diluted appropriately when used.

About the use of balance buffer

1. Description

   The nucleic acid adsorption silica gel membrane column reacts with the charge / dust in the air during long-term placement, which affects its nucleic acid binding ability. After pretreatment with balance buffer, the hydrophobic groups of silica gel membrane in the column can be greatly reduced and the binding ability of nucleic acids can be improved. So as to improve the recovery efficiency or output of silica gel column. Balance buffer is a strong alkaline solution. If it is accidentally touched, please clean it with plenty of tap water. Close the bottle cap after use to avoid contact with air. Store at room temperature. There may be precipitation during storage. Please heat it to 37℃ to completely eliminate the precipitation.
2. Protocol

   Take a new silica gel membrane adsorption column and install it in the collection tube, suck 100μL balance buffer into the column. Centrifuge at 13000 rpm for 1 minute, pour out the waste liquid in the collection pipe, and put the adsorption column back into the recovery header. At this time, the balance buffer pretreatment column is completed. Follow up operation steps.

Protocol(Please read the note before the experiment)

Tips: 1) before using for the first time, please add the specified amount of absolute ethanol into the buffer WB bottle and mix well. After adding, please tick the box to mark that ethanol has been added in time, so as to avoid adding it many times!

2)Add all RNase A into 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, drain the supernatant as much as possible, and collect the bacteria.
2. Resuspend the bacterial pellet with 500μl buffer P1, vortex it until it is completely suspended, and transfer it all into a 2ml centrifuge tube.

   If there is a bacteria block that is not thoroughly mixed, it will affect the lysis, resulting in low extraction amount and purity.
3. Add 500μl of buffer P2, gently flip it up and down for 6-8 times to fully lyse the bacteria, and leave it at room temperature for 4 minutes.

   Mix gently and do not shake violently to avoid genomic DNA shearing and breaking! The time should not exceed 5 minutes! To avoid plasmid damage. At this time, the bacteria liquid should become clear and viscous. If there are few bacteria, the next step can be done soon after clearing and viscous, not necessarily accurate for 5 minutes.
4. Add 500μl buffer N3, turn it up and down gently for 6-8 times immediately, and white flocculent sediment will appear when it

is fully mixed. Centrifuge at 13000rpm for 10 minutes and carefully take the supernatant to a new tube to avoid absorbing the floating white sediment.

5. Add 0.1 volume (10% of the volume of supernatant, about 160μl) of buffer Er to the supernatant obtained in the previous step, rotate upside down and mix well, place in ice bath or insert into crushed ice (or refrigerator freezer) for 5 minutes until the turbidity becomes clear and transparent (or still slightly turbid), and mix occasionally in the middle.

   After buffer Er is added to the supernatant, the supernatant will become turbid, but it should be clear (or slightly turbid) after ice bath.

6. Place it at room temperature for 3-5 minutes, and the solution will soon become turbid when the temperature returns to room temperature, and mix it upside down.

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

7. Centrifuge at 14000×g for 10 min at room temperature for phase separation. The upper aqueous phase contains DNA, and the lower blue oily phase contains endotoxin and other impurities. Transfer the upper aqueous phase containing DNA to a new tube (be careful not to absorb impurities such as endotoxin in the blue oily layer), and discard the oily layer.

   Balance buffer pretreatment adsorption column: pretreatment of silica gel membrane adsorption column with balance buffer is a necessary step. For specific methods, see "about the use of balance buffer" above

8. Add 0.5 volume of isopropanol (about 740μl) to the upper aqueous phase, fully mix it upside down, and transfer it into the adsorption column AC several times (no more than 700μl each time) (the adsorption column is put into the collection tube), centrifuge at 12000×g for 1 minute, and pour out the waste liquid in the collection tube. Until all mixed solutions pass through this adsorption column.

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

   In order to remove trace nuclease and other impurities, this step should be added if the strains used are endo strains such as JM series and HB101 or wild-type strains with abundant nuclease content; If the strains used were XL-1 blue, TOP10 and DH5 α If the nuclease content of other defective strains is low, this step can be skipped.

10. Add 600μl buffer WB (please check whether absolute ethanol has been added!), 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 12000rpm for 2 minutes, and try to remove buffer WB to avoid residual ethanol in buffer WB inhibiting the downstream reaction.

12. Take out the adsorption column AC, put it into a clean centrifuge tube, add 100-200μl buffer EB in the middle of the adsorption membrane (buffer EB is better heated in 65-70 ℃ water bath in advance), place it at room temperature for 2 minutes, and centrifuge at 12000rpm for 1 minute. If a large amount of plasmid is needed, the obtained solution can be added to the centrifugal adsorption column again, placed at room temperature for 2 minutes, and centrifuged for 1 minute.

    The larger the elution volume, the higher the elution efficiency. If the plasmid concentration is high, the elution volume can be appropriately reduced, but the minimum volume should not be less than 100μl. Too small volume reduces the efficiency of plasmid elution and plasmid yield.