Yeast plasmid small extraction kit

Item No.: HDP207 100 times
HDP207M 200 times

Kit composition	100 Time	200 time
RNaseA（10mg/ml）	300 μl	300 μl
Solution YS1	25 ml	50ml
Solution YS1	25 ml	50ml
Solution YS3	40 ml	80 ml
Rinsing liquid PE	50 ml	100 ml
	25 ml	50 ml
	Add the specified amount of ethanol according to the instructions before the first use	
Elution buffer EB	10 ml	20 ml
Lysozyme	50 mg	100mg
Adsorption column AC	100A	200A
Collection tube (2ml)	100A	200A

Storage matters:

1. When using for the first time, add all RNase A included in the kit to solution YS1 (final concentration 100ug/ml) and store at 2-8°C. If RNase A in solution YS1 is inactivated, there may be traces of RNA remaining in the extracted plasmid, so add RNase A to solution YS1.
2. When the ambient temperature is low, the solution YS2 may precipitate turbidity or precipitation. It can be heated in a 37°C water bath for a few minutes to recover its clarification.
3. Avoid long-term exposure of the reagents to the air to cause volatilization, oxidation, and pH changes. The lid should be closed in time after each solution is used.

Need to bring your own reagents:

Sorbitol buffer: use 0.1M sodium phosphate buffer (pH7.4) to prepare 1.2 M sorbitol; 0.1M sodium phosphate buffer (pH7.4) to prepare: 77.4 ml 0.1 mol/L Na 2 HPO 4 + 22.6ml 0.1 mol/L NaH 2 PO4, take 15 ml of sorbitol buffer to dissolve lysozyme, or add it as needed for each test.

Product description:

This product uses an improved SDS-alkaline lysis method to lyse cells. The silicon matrix membrane in the centrifugal adsorption column combines with the plasmid DNA in the solution under high salt and low pH conditions, and then removes impurities and other bacterial components through deproteinization solution and rinse Removal, and finally a low-salt, high-pH elution buffer will elute the pure plasmid DNA from the silicon matrix membrane.

Features:1. The silicon matrix membrane in the centrifugal adsorption column all adopts imported special adsorption membrane, the adsorption amount difference between the column and the column is very small, and the repeatability is good.
2. The unique protein-removing liquid formula can efficiently remove the residual nuclease, even the strains with rich nuclease content such as JM series and HB101 can also be easily removed. Effectively prevent the plasmid from being degraded by nuclease.
3. Fast and convenient, no need to use toxic phenol, chloroform and other reagents, and no need for ethanol precipitation. The obtained plasmid has high yield and good purity, and can be directly used in various molecular biology experiments such as restriction digestion, transformation, PCR, in vitro transcription, and sequencing.
Precautions
a. All centrifugation steps are completed at room temperature, and the centrifuge speed needs to reach 13,000 rpm.
b. The obtained plasmid DNA can be tested for concentration and purity by agarose gel electrophoresis and UV spectrophotometer. An OD260 value of 1 is equivalent to approximately 50 μg/ml DNA. The electrophoresis may be a single band, or it may be 2 or more DNA bands. This is mainly caused by different degrees of supercoiled conformation plasmid migrating positions, which is related to the length of the extract culture time and the severity of the operation during extraction. . The basic super spiral can exceed 90% under normal operation of the company's products.
c. The exact molecular size of plasmid DNA must be digested and linearized, and then the DNA molecular weight Marker can be compared. Plasmids in a circular or supercoiled state have an indeterminate migration position, and the exact size cannot be known by electrophoresis.
d. The eluent EB does not contain the chelating agent EDTA, which does not affect downstream digestion, connection and other reactions. Water can also be used for elution, but it should be ensured that the pH is greater than 7.5. If the pH is too low, the elution efficiency will be affected. Plasmid eluted with water should be stored at -20°C. If the 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, so it can be diluted appropriately during use.
Operation steps: (please read the precautions before the experiment)
Prompt:
ð Please add the specified amount of absolute ethanol to the rinsing solution WB and deproteinization solution PE bottle before the first use, and mix them thoroughly. After adding, please tick the box to mark that ethanol has been added in time to avoid multiple additions!
ð Add all RNase A to solution YS1, mix well, and store at 2-8°C after each use.
1. Take 1-5 ml of yeast culture, centrifuge at 12,000 rpm (~13,400×g) for 1 min, and aspirate the supernatant as much as possible.
2. Add 300 μl of sorbitol buffer to the bacteria, add about 0.5 mg of lysozyme, and mix well. Treat at 30°C for 30 min. Centrifuge at 4000 rpm (~1500×g) for 10 min, discard the supernatant, and collect the precipitate.
3. Resuspend the bacterial pellet with 250μl solution YS1, and vortex until it is completely suspended.

If there are bacteria clumps that are not thoroughly mixed, it will affect the lysis, resulting in low extraction volume and purity.

4. Add 250μl of solution YS2, gently turn up and down 6-8 times to fully lyse the bacteria, and leave it at room temperature for 4 minutes.

Mix gently, do not shake vigorously to avoid shearing and breaking of genomic DNA! The time used should not exceed 5 minutes! So as not to damage the plasmid. At this time, the bacteria liquid should become clear and viscous. If there are few bacteria, the next step can be done after the bacteria are clear and viscous. It does not have to be accurate for 5 minutes.

5. Add 350μl of YS3 solution, and immediately turn it upside down gently 6-8 times. A white flocculent precipitate will appear when thoroughly mixed. Centrifuge at 13,000 rpm for 10 minutes, and carefully remove the supernatant.

After adding solution YS3, it should be mixed immediately to avoid local precipitation of SDS.

6. Add the supernatant obtained in the previous step to the adsorption column AC (the adsorption column is placed in the collection tube), centrifuge at 12,000 rpm for 30-60 seconds, and discard the waste liquid in the collection tube.

7. Optional step: add 500μl of protein-removing solution PE, centrifuge at 12,000rpm for 30-60 seconds, and discard the waste solution.

This step is to remove trace amounts of nuclease and other impurities. If the strain used is endA strain such as JM series, HB101 or wild-type strain, which contains abundant nuclease, this step should be added; if the strain used is XL-1 Blue, Top10 and DH5α, etc. Defective strains with low nuclease content can skip this step.

8. Add 600μl of rinsing solution WB (please check if absolute ethanol has been added first!), centrifuge at 12,000 rpm for 30 seconds, and discard the waste solution.

9. Add 600μl of rinsing solution WB, centrifuge at 12,000 rpm for 30 seconds, and discard the waste solution.

10. Put the adsorption column AC back into the empty collection tube and centrifuge at 13,000 rpm for 2 minutes to remove the rinsing liquid as much as possible, so as to avoid residual ethanol in the rinsing liquid from inhibiting downstream reactions.

11. Take out the adsorption column AC, put it into a clean centrifuge tube, add 50-100μl elution buffer EB to the middle part of the adsorption membrane (the elution buffer is heated in a 65-70℃ water bath in advance for better effect), Leave at room temperature for 2 minutes, and centrifuge at 12,000 rpm for 1 minute. If a larger amount of plasmid is needed, add the obtained solution to the centrifugal adsorption column and centrifuge for 1 minute.

The larger the elution volume, the higher the elution efficiency. If a higher plasmid concentration is required, the elution volume can be appropriately reduced, but the minimum volume should not be less than 30μl. Too small a volume reduces the efficiency of plasmid elution and reduces the yield of plasmid.