Hligene Yeast mini plasmid purification kit

Code No.: NG207S 100T

NG207M 200T

Contents	storage	100 T	200 T
RNaseA (10 mg/ml)	-20 ℃	250 µl	500 µl
lysozyme	4 ℃	50 mg	100 mg
Buffer YS1	4 ℃	25 ml	50 ml
buffer YS2	RT	25 ml	50 ml
buffer YS3	RT	40 ml	80 ml
buffer PE	RT	50 ml	100 ml
Buffer WB	RT	25 ml	50 ml
		Add absolute alcohol before use	
Spin Columns AC	RT	100	200
Buffer EB	RT	10 ml	20 ml
Collection tubes(2ml)	RT	100	200

Storage:

1. At first use, all the RNase A in the kit was added to buffer YS1 and stored at 2-8 °C.
2. All solutions should be clear, but it may form precipitates if temperature is low, can be heated in a 37 °C water bath for a few minutes to restore clarity.
3. Avoid reagent exposure to the air for a long time , which could be volatilization, oxidation, pH changes, the solution should be timely close the lid after use.

Reagents (provided by yourself);

Sorbitol buffer: 1.2 M sorbitol prepared with 0.1 M sodium phosphate buffer (pH 7.4): 77.4 ml of 0.1 M sodium phosphate buffer (pH 7.4) and 22.6 ml of 0.1 mol/L NaH2PO4; take 30 ml of sorbitol buffer to dissolve lysozyme in the 100-test kit, and so on, or add as needed for each experiment.

Introduction:

The modified SDS-alkaline lysis was used to lyse the cells, the new lysis buffer allows the adsorption of DNA onto silica membrane in the presence of high salt., and then the impurities and other bacterial components were removed by rinsing solution, finally, the purified plasmid DNA was eluted from the silica matrix membrane by low salt and high pH elution buffer.

Product Specifications:

1. The adsorption amount among the columns is very small, and the repeatability is good.

2. PE can effectively remove the residual nuclease, even the nuclease-rich strains such as JM series, HB101 can be easily removed.
3. The kit is fast and convenient, without the use of toxic phenol, chloroform and other reagents, nor the need for ethanol precipitation. The obtained plasmid has high yield and purity which can be directly used in molecular biology experiments such as enzyme digestion, transformation, PCR, in vitro transcription, sequencing and so on.

Safety Information:

1. All centrifugation steps are performed at room temperature and the centrifuge speed is required to reach 13,000 rpm.
2. The amount of plasmid was related to bacterial culture concentration and plasmid copy number. In general, 20μg plasmid can be isolated from 1.5-4.5 ml high-copy plasmids which inoculated on LB with suitable antibiotics and cultured for 14-16 hours. If the plasmids are low copy or larger than 10kb, the amount of bacteria should be increased appropriately, and the amount of S1, S2 and S3 should be increased proportionally.
3. The buffer EB did not contain EDTA. Water can also be used, but it should be ensured that pH is greater than 7.5. The plasmids should be stored at ~ 20 °C. Plasmid DNA can be eluted with TE buffer (10 mm Tris-HCl, 1 mm EDTA, pH 8.0) if long-term preservation is required, but EDTA may affect downstream enzymatic digestion reactions.

Operation Procedure:

Tip: before the first use, please add absolute alcohol in the buffer WB and mark it.

Ensure that RNase A have been added to Buffer YS1， then store at 4 °C.

1. Harvest 1-5 ml bacterial cells in a microcentrifuge tube by centrifugation at 12,000 rpm for 1 min at room temperature (15-30 °C), then remove all traces of supernatant until all medium has been drained.
2. Resuspend pelleted bacterial cells in 300 μl Sorbitol buffer by Vortex oscillates to complete suspension, Placed at 30 °C for 30 min. Centrifugation at 4,000 rpm for 10 min, then remove all traces of supernatant until all medium has been drained.
3. Resuspend pelleted bacterial cells in 250 μl Buffer YS1 by Vortex oscillates to complete suspension.
   Note: Cell clumps indicate incomplete lysis, which will result in lower yield and purity.
4. Add 250 μl Buffer YS2 and mix gently turned up and down 6-8 times to lyse fully. Placed at room temperature for 4 min.
   Note: Mix gently by inverting the tube. Do not vortex, as this will result in shearing of genomic DNA. If necessary, continue inverting the tube until the solution becomes viscous and slightly clear. If not clear, probably due to incomplete lysis, please reduce the cells.
5. Add 350 μl buffer YS3, immediately turn up and down gently 6-8 times, when fully mixed, the solution should become cloudy. Centrifuge at 12,000 rpm for 10 min and carefully remove the supernatant.
   Note: To avoid localized precipitation, mix the solution quickly, immediately after addition of Buffer YS3.

6. Transfer the supernatant from step 4 to the Spin Column AC (put in a Collection Tube) by pipetting. Centrifuge for 30 s at 12,000 rpm. Discard the flow-through and set the Spin Column AC back into the Collection Tube.
7. Add 500 µl of Buffer PE to the Spin Column and centrifuge for 30 sec, 12,000 rpm, Discard the flow-through and place the Spin Column back in the same collection tube.
8. Add 600 µl of Buffer WB to the Spin Column and centrifuge for 30 sec, 12,000 rpm, Discard the flow-through and place the Spin Column back in the same collection tube.
9. Add 600 µl of Buffer WB to the spin column and centrifuge for 30 sec, 12,000 rpm again, Discard the flow-through and place the Spin Column back in the same collection tube, centrifuge for an additional 2 min, 12,000 rpm.
10. Place the Spin Column into a clean 1.5 ml microcentrifuge tube.
11. Add 50-100 µl of Buffer EB or water to the center of the membrane, let the column stand for 1 min, and then centrifuge for 1 min at 12,000 rpm（The elution buffer can be preheated at 65-70 °C to increase the elution effect）.

    Note: The volume of elution buffer should not be less than 30 µl, otherwise it may affect recovery efficiency. The pH value of elution buffer will have a great effect on eluting.