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Yeast Genomic DNA Kit

Product Number: DNK2801

Shipping and Storage

1. When the ambient temperature is low, some detergent ingredients in Buffer YL will precipitate and become turbid or precipitate. It can be heated in a 37℃ water bath for a few minutes and gently shaken to restore clarity. Do not shake violently to avoid excessive foam formation.
2. Buffer PP may experience precipitation and precipitation, and can be re dissolved by taking a water bath at 37℃ for a few minutes. If it cannot be completely dissolved, it will not affect the effectiveness of use. Simply take the upper solution.
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	DNK2801 50preps	DNK2802 100preps	DNK2803 200preps
Buffer YL	RT	15 ml	30ml	60ml
Buffer PP	RT	5 ml	10ml	20ml
Buffer DD	RT	5 ml	5ml	10ml

Description

This kit is used for rapid extraction of genomic DNA from yeast. Under the action of Buffer YL formulated based on the characteristics of yeast cells, yeast cells are lysed to release genomic DNA, which is then selectively precipitated by Buffer PP to remove proteins. Finally, pure genomic DNA is precipitated in isopropanol and re dissolved in Buffer DD.

Features

1. No need to use toxic reagents such as phenol and chloroform.
2. Fast and simple, the entire process can be completed within 1 hour.
3. The results are stable and the yield is high (more than twice that of centrifugal column type), with a typical OD260/0D280 ratio of 1.7~1.9 and a length of 50Kb-150kb. It can be directly used for PCR, Southern blot, various enzyme digestion reactions, and library construction.

Protocol

1. Suck 1.5ml of yeast culture into a 1.5ml centrifuge tube; Centrifuge at 12000rpm for 2 minutes, discard the supernatant as much as possible, and if necessary, use a gun to remove it.
2. High speed vortex oscillation disperses resuspended yeast cell clusters.
3. Add 300μl Buffer YL, vortex shake and mix well, or repeatedly blow and mix with a 1ml gun tip.
   The resuspension and dispersion of yeast cells are crucial for the next step of lysis and must be fully dispersed and resuspended.
4. Place the lysate in a 70℃ water bath for 15-30 minutes.
   If the yield is low, the water bath temperature can be appropriately increased and the water bath time can be extended. In the middle, vortex oscillation can be mixed several times to help with cracking.
5. Let it recover to room temperature on ice for at least 5 minutes.
6. After adding 100μl of Buffer PP to the pyrolysis product that has returned to room temperature, shake continuously at high speed on a vortex oscillator and mix well for 20 seconds. After mixing, you may see some small protein clumps. Ice bath for 5 minutes.

7. Centrifuge at 13000rpm for 5-10 minutes. At this point, protein precipitates at the bottom of the tube should be visible, and some protein precipitates may also be seen floating on the surface of the liquid.
8. Carefully and slowly aspirate the supernatant into a new 1.5ml centrifuge tube, do not aspirate the sediment.

   When aspirating the supernatant, be careful not to aspirate the protein precipitate at the bottom of the tube or floating on the surface of the liquid. If the protein precipitate is accidentally transferred into a new centrifuge tube, it can be centrifuged again for 2 minutes before taking the supernatant.
9. Add an equal volume of room temperature isopropanol (about 400μl), invert 30 times and mix well until flocculent DNA precipitate (or white turbid precipitate) appears.
10. Centrifuge at 12000 rpm for 1 minute, and white DNA precipitates can be seen at the bottom of the tube. Discard the supernatant.
11. Add 1ml of 70% ethanol, invert and rinse the DNA precipitate several times, centrifuge at 12000rpm for 1 minute, remove the supernatant (be careful not to pour out the DNA precipitate), invert and gently tap on absorbent paper a few times to control the residual ethanol. You can also use a gun to carefully suck out the residual ethanol around the bottom precipitate and the wall of the tube, and air dry the precipitate for a few minutes.

    Be careful not to dry too much, otherwise DNA is extremely insoluble; Also, too much ethanol should not be left behind, otherwise ethanol may inhibit downstream reactions such as enzyme digestion.
12. Add 40μl of Buffer DD to rehydrate and dissolve the DNA precipitate, gently flick the tube wall and mix well. It can be incubated at 65℃ for 30-60 minutes (not more than one hour), or left overnight at room temperature or 4℃ to rehydrate the DNA. During this period, occasional light tapping of the tube wall helps rehydrate DNA.
13. Add 10-15μl of RNase A (10mg/ml) or 1-2μl of RNase A (100mg/ml) upside down and mix well. Incubate at 37℃ for 30-60 minutes to remove residual RNA.

    The main function of this step is to remove residual RNA. If there is a lot of residual RNA, it can be left for an appropriate amount of time. If residual RNA does not affect the experiment, this step can be omitted. If the residual RNA enzyme may affect the experiment, it can also be removed by equal volume phenol/chloroform extraction, and then DNA can be recovered by precipitation with standard ethanol.
14. DNA can be stored at 2-8℃, and if it needs to be stored for a long time, it can be placed at -20℃.