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

Yeast Genomic Medi Kit

Product Number: DNK2901

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	DNK2901 10preps
Buffer YL	RT	100ml×2
Buffer PP	RT	70ml
Buffer DD	RT	30ml

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/OD280 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. Take 60ml-70ml of yeast culture into a 100ml centrifuge tube; Centrifuge 2500×g for 2 minutes and discard the supernatant as much as possible. If necessary, use a gun to remove it.
2. High speed vortex oscillation disperses resuspended yeast cell clusters.
3. Add 20 ml of Buffer YL, vortex shake and mix well, or repeatedly blow and mix with a 1ml nozzle.
   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. At least 5 minutes on ice to restore to room temperature,
6. Add 6.8ml of Buffer PP to the pyrolysis product that has been restored to room temperature, and mix continuously on a vortex oscillator for 25 seconds at high speed. After mixing, you may see some small protein clumps. Ice bath for 5 minutes.
7. 2.500×g (centrifugal force can be adjusted and increased as needed) Centrifuge for 5 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 100ml 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, invert 30 times and mix well until flocculent DNA precipitate (or white turbid precipitate) appears.
10. Centrifuge 2.500×g for 5 minutes (centrifugal force can be adjusted as needed), and white DNA precipitates can be seen at the bottom of the tube. Discard the supernatant.
11. Add 20ml of 70% ethanol, invert and rinse the DNA precipitate several times, centrifuge 2500×g for 2 minutes, pour out 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 1-3ml Buffer DD to rehydrate and dissolve the DNA precipitate. Gently bounce 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 0.5-1ml RNase A (10mg/ml), mix upside down, and 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 large amount of residual RNA, the time can be appropriately extended or the amount of RNase A can be increased. 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/mimetic 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 ℃.