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

Tissue/cell genomic DNA Extraction Kit

Product Number: DNK0801

Shipping and Storage

1. When the ambient temperature is low, some detergent ingredients in Buffer NLY will precipitate and become turbid or precipitate. 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.
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	DNK0801 50 Preps	DNK0802 100 Preps	DNK0803 200 Preps
Buffer NLY	RT	30 ml	60 ml	120 ml
Buffer PP	RT	10 ml	20 ml	40 ml
Buffer DA	RT	10 ml	15 ml	30 ml
RNase A(10mg/ml)	-20℃	100 μl	200 μl	400 μl

Description

This kit is used for rapid extraction of genomic DNA from plant cells/tissues. After grinding or homogenizing the sample, buffer NLY is added. Firstly, cells are lysed under strong detergents or in synergy with Proteinase K to release genomic DNA. Then, RNase A is added to remove RNA, followed by selective precipitation of buffer PP to remove protein. Finally, pure genomic DNA is precipitated in isopropanol and re dissolved in buffer DA.

Features

1. No need to use toxic reagents such as phenol and chloroform.
2. Fast and simple, the entire process of organizing sample operations 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.

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to 13000rpm.
2. Users need to provide isopropanol, 70% ethanol, PBS (for cells), liquid nitrogen mortar/or homogenizer (for tissues), 0.5M EDTA and Proteinase K (for mouse tail), and a water bath.
3. Preheat the required water bath before starting the experiment for later use.
4. This reagent kit is a solution type and can easily increase or decrease the amount of tissue cells processed each time in proportion.

Protocol(Please read the precautions before the experiment)

1. Tissue cultured cells

   1.1. Collect cells into a 1.5ml centrifuge tube; For adherent cells, they should be digested with trypsin first and then collected

by blowing them down.

1.2. Centrifuge at 13000rpm for 10 seconds to allow the cells to precipitate. Discard the supernatant, leaving behind cell clusters and approximately 10-50µl of residual liquid.

1.3. Add 200µl PBS to resuspend and wash the cells, repeat the previous step, and resuspend the cell clusters with high-speed vortex oscillation.

1.4. For cell lines with poor buffer NLY lysis efficiency (such as PC12 cells), a few freeze-thaw cycles should be performed before the next step: freeze in liquid nitrogen, melt in a 95℃ water bath, and repeat 4 times.

1.5. Add 600µl Buffer NLY and gently blow the lysed cells with a large caliber gun tip (cutting off the tip) until no cell clumps are visible.

1.6. Follow step 4 of the operation.

2. Animal and plant tissues (such as mouse liver and brain or plant leaves)

2.1. Add 10-20mg of fresh or thawed tissue to 600µl pre cooled buffer NLY ice, homogenize with a small homogenizer for 10 seconds, and transfer the lysate into a 1.5ml centrifuge tube. Another method: Grind 10-20mg of tissue in liquid nitrogen (plant leaves can be added as much as 40mg) to form a fine powder, then transfer it to a 1.5ml centrifuge tube containing 600µl of pre cooled buffer NLY ice, and mix well with a large caliber gun.

2.2. Place the lysate in a 65℃ water bath for 15-30 minutes.

2.3. Follow step 4 of the operation.

3. Animal tissue (rat tail)

3.1. Before processing the sample, add 120µl 0.5M EDTA (pH 8.0) to a 1.5ml centrifuge tube containing 500µl Buffer NLY, mix well, and pre cool with ice for later use.

3.2. Grind the mouse tail into fine powder in liquid nitrogen or cut the tip of the mouse tail from 0.5 to 1.0cm (be sure to cut the tip within the range of 0-2cm, otherwise the cracking effect will not be good) into small pieces and place it in a 1.5ml centrifuge tube. Then, add 600µl of EDTA/Buffer NLY prepared in the previous step.

3.3. Add 17.5µl of Proteinase K solution (20mg/ml), invert and mix well.

3.4. Leave the 55℃ water bath overnight and gently shake it a few times to aid in cracking. Alternatively, take a water bath at 55℃ on a shaker for 3 hours and mix thoroughly with high-speed vortex oscillation every hour. Ensure that the tail of the mouse is completely cleaved (uncut tail may not be completely cleaved and yield may be lower).

4. Add 1.8µl of RNase A (10mg/ml) to the lysate, with a final concentration of 30µg/ml. Invert and mix well, then incubate at 37℃ for 15-30 minutes to remove residual RNA. Then cool to room temperature for at least 5 minutes or take an ice bath to restore to room temperature.

5. Add 200µl Buffer PP to the pyrolysis product that has returned to room temperature, and then shake continuously at high speed on a vortex oscillator for 25 seconds to mix well. After mixing, you may see some small protein clumps. Ice bath for 5 minutes.

Due to the small volume and weight of the sample, the shear force generated by shaking and mixing with a vortex oscillator will not interrupt the genomic DNA. If you shake and mix with your hands, you cannot shake vigorously up and down with your hands. You can only shake and mix with appropriate force, otherwise it will cut the genomic DNA; However, the force should not be too small. It is necessary to ensure thorough mixing and disperse the viscous lysate. Otherwise, DNA cannot separate from the egg's cytoplasmic precipitate. When centrifuged, it will precipitate together with the protein, causing DNA loss or reduced yield.In addition, insufficient mixing may also result in insufficient protein precipitation, and the final product may contaminate a large amount of protein. Therefore, it is recommended to use a vortex oscillator.

6. Centrifuge at 13000rpm 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.

7. 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, the supernatant can be taken after centrifugation for another 2 minutes

8. Add an equal volume of room temperature isopropanol (approximately 600μl), invert 30 times and mix well or until a cotton like filamentous white DNA precipitate appears.

   Note:When mixing upside down, the cotton like filamentous DNA sometimes adheres to the lid or tube opening, and even if it is upside down, it does not follow. This can cause the operator to not see the sediment and mistakenly believe that DNA has not been obtained. The solution is to skip step 9, centrifuge at 12000 rpm for 1 minute, discard the supernatant, and then proceed to step 11.

9. Place the centrifuge tube vertically to allow the white DNA precipitate to naturally settle to the bottom of the tube, and then absorb as much of the supernatant as possible, being careful not to inhale the precipitate.

   If there are bubbles attached to the cotton like filamentous DNA precipitate, it will float on the surface of the liquid and will not precipitate. Be careful not to absorb the precipitate from the supernatant.

10. After adding 1ml of 70% ethanol, invert and rinse the DNA precipitate. Centrifuge at 12000rpm for 1 minute, and white DNA precipitate blocks 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, pour out the supernatant (be careful not to pour out the DNA precipitate), invert and gently dry the residual ethanol on absorbent paper a few times. 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.

    Note:Do not dry too much, otherwise DNA is insoluble and too much ethanol should not be left behind, otherwise ethanol may inhibit downstream reactions such as enzyme digestion.

12. Add 100μl of Buffer DA to rehydrate and dissolve the DNA precipitate. Mix the lightly bounced tube wall well and incubate at 65℃ for 30-60 minutes (no more than one hour). During this time, gently bounce the tube wall to help rehydrate the DNA. DNA can also be rehydrated by staying overnight at room temperature or 4℃.

13. DNA can be stored at 2-8℃, and if it needs to be stored for a long time, it can be placed at -20℃.