

Genomic Medium Kit

Product Number: DNK0901

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°C 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°C 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	DNK0901	DNK0902
		20 Preps	50 Preps
Buffer NLY	RT	180 ml	450 ml
Buffer PP	RT	60 ml	150 ml
Buffer DA	RT	10 ml	20 ml
RNase A(10mg/ml)	-20°C	360 µl	900 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 Protocols 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.

Application

Suitable for rapid extraction of genomic DNA from various animal and plant cells/tissues

Note

1. All centrifugation steps are completed at room temperature using a traditional desktop centrifuge with a speed of up to 3000×g.
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)

For Research Use Only

1. Tissue cultured cells

- 1.1. Collect $6-8 \times 10^7$ cells into a 50ml centrifuge tube; For adherent cells, they should be digested with trypsin first and then collected by blowing them down.
- 1.2. Centrifuge $500 \times g$ for 5 minutes to allow the cells to precipitate. Discard the supernatant, leaving behind cell clusters and approximately 100 μ l of residual liquid.
- 1.3. Add 3ml 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°C water bath, and repeat 4 times.**
- 1.5. Add 9ml of Buffer NLY and gently blow the lysed cells with a large caliber gun (cutting off the tip of the gun) until no cell clumps are visible.
- 1.6. Follow step 4 of the Protocol.

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

- 2.1. Add 9ml of pre cooled buffer NLY to 300mg of fresh or thawed tissue, homogenize completely in a homogenizer, and transfer the lysate into a 50ml centrifuge tube. Another method is to grind 300mg of tissue into fine powder in liquid nitrogen, transfer it into a 50ml centrifuge tube containing 9ml of pre cooled buffer NLY ice, and mix well with a large caliber gun.
- 2.2. Place the lysate in a 65°C water bath for 15-30 minutes.

If maximum yield is required, 45 μ l of protease K (20mg/ml) can be added, mixed 25 times upside down, and incubated at 55°C for 3 hours or overnight. Until the tissue dissolves, occasionally mix upside down in the middle.

- 2.3. Follow step 4 of the Protocol.

3. Plant tissue

- 3.1. After grinding plant tissue (200mg dry tissue or 400mg wet tissue) into fine powder in liquid nitrogen, transfer it into a 50ml centrifuge tube containing 9ml of pre cooled buffer NLY ice, and use a large caliber gun to blow and mix well.

The initial processing capacity of plant tissue should be adjusted based on leaf age, species, and genome size.

- 3.2. Place the lysate in a 65°C water bath for 15-60 minutes, inverting it at least 10 times during this period.

4. Add 18 μ l of RNase A (10mg/ml) to the lysate, with a final concentration of 30 μ g/ml. After mixing 25 times, incubate at 37°C for 15-60 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 3ml 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.
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 $2500 \times g$ for 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.
7. Carefully and slowly aspirate the supernatant into a new 50ml 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 another 2 minutes of centrifugation.
8. Add an equal volume of room temperature isopropanol (about 9ml), 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 2000×g directly for 3-5 minutes, 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 9ml of 70% ethanol, invert and rinse the DNA precipitate. Centrifuge 2000×g for 3-5 minutes, and white DNA precipitate blocks can be seen at the bottom of the tube. Discard the supernatant.
11. Add 5ml of 70% ethanol, invert and rinse the DNA precipitate several times, centrifuge 2000×g 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 500μl of Buffer DA to rehydrate and dissolve the DNA precipitate. Mix the lightly bounced tube walls evenly and incubate at 65°C for 60 minutes (not more than one hour). During this time, gently bounce the tube walls to help rehydrate the DNA. DNA can also be rehydrated by staying overnight at room temperature or 4°C.
13. DNA can be stored at 2-8°C, and if it needs to be stored for a long time, it can be placed at -20°C.