Cell / tissue genomic DNA extraction kit (solution type)

Item No.: HPR402 100 times
HPR402M 200 times

Kit composition	**Save**	**100 Times**	**200 Times**
Nuclear lysate	Room Temperature	60 ml	120 ml
Protein precipitation solution	Room Temperature	20 ml	40 ml
DNA dissolving solution	Room Temperature	15 ml	30 ml
RNase A(10 mg/ml)	-20℃	200 μl	400 μl
Filter column	Room Temperature	100Set	200Set

1. When the ambient temperature is low, some detergent components in the nucleus lysate will precipitate and appear turbid or precipitate. You can heat it in a 37 ° C water bath for a few minutes and gently swirl to restore clarification. Do not shake vigorously to avoid excessive formation. Foam.
2. The protein precipitation solution may precipitate and precipitate. It can be re-dissolved in a 37 ° C water bath for a few minutes. If it cannot be completely dissolved, it will not affect the use effect, just take the upper solution directly.
3. Avoid long-term exposure of the reagents to the air to cause volatilization, oxidation, and pH changes. The lid should be closed in time after each solution is used.

Product description:

This kit is used to quickly extract genomic DNA from animal and plant cells / tissues. After the sample is ground or homogenized, the nucleus lysis solution is added. First, the cells are lysed with a strong detergent or under the synergistic action of proteinase K to release genomic DNA, then RNase A is added to remove RNA, and then the protein precipitation solution is selectively precipitated to remove proteins, and finally purified The genomic DNA is precipitated with isopropanol and re-dissolved in the DNA dissolving solution.

Features:

1. No need to use toxic phenol, chloroform and other reagents.
2. Fast and simple, the entire process of organizing sample operations can be completed within 1 hour.
3. The result is stable and the yield is high (more than twice the yield of the spin column type). The typical ratio of OD260 / OD280 is 1.7 ～ 1.9, and the length can reach 50Kb-150kb. It can be

used directly in PCR, Southern blot and various enzyme digestion reaction and library construction.

Precautions

a. All centrifugation steps are completed at room temperature, and the speed of the centrifuge must reach 13,000 rpm.

b. Users need to bring their own isopropanol, 70% ethanol, PBS (for cells), liquid nitrogen mortar / or homogenizer (for tissues), 0.5 M EDTA and proteinase K (for rat tail), water bath box.

c. Before starting the experiment, preheat the required water bath for later use.

Operation steps: (please read the precautions before the experiment)

ð Tissue culture cells

1. Collect the cells into a 1.5 ml centrifuge tube; for adherent cells, trypsinization should be used first and then pipetting down to collect.

2. Centrifuge at 13,000 rpm for 10 sec to precipitate the cells. Discard the supernatant, leaving the cell cluster and approximately 10-50 µl of residual liquid.

3. Add 200 µl PBS to resuspend the washed cells, repeat the previous step, vortex at high speed to resuspend the cell cluster.

4. For cell lines whose nuclear lysate does not lyse well (such as PC12 cells), you should do several freeze-thaw cycles before proceeding to the next step: after freezing in liquid nitrogen

Melt in a water bath at 95 ° C and repeat 4 times.

5. Add 600 µl of cell nuclear lysate, and use a large-caliber pipette tip (cut off the tip of the pipette tip) to gently lyse the cells until no cell clumps are visible.

6. Go to step 4 under operation steps.

ð Animal and plant tissues (such as rat liver and brain or plant leaves)

1. Add 10-20 mg of fresh or thawed tissue to 600 µl of ice-cold nuclear lysate, homogenize with a small homogenizer for 10 sec, and transfer the lysate to a 1.5 ml centrifuge tube. Another method: Grind 10-20 mg of tissue in liquid nitrogen (plant leaves can be appropriately added, such as 40 mg) into fine powder, and transfer it to a 1.5 ml centrifuge tube containing 600 µl of ice-cold nuclear lysate. Use a large-caliber pipette tip to blow and mix well.

2. Place the lysate in a 65 ℃ water bath for 15-30 min.

3. Go to step 4 under the operation steps.

ð Animal tissue (rat tail)

1. Before processing the sample, add 120µl 0.5 M EDTA (pH 8.0) to a 1.5ml centrifuge tube containing 500µl nuclear lysate, mix well and pre-cool on ice for later use.

2. Grind the tail of the rat into fine powder in liquid nitrogen or cut the tail tip of 0.5-1.0cm (be sure to cut the tip of the tail within the range of 0-2cm, otherwise the lysis effect will not be good) and cut it into a 1.5 ml centrifuge After the tube, add 600 µl of the prepared EDTA / nucleus lysate.

3. Add 17.5 µl proteinase K solution (20 mg / ml) and mix by inversion.

4. Place it in a 55 ℃ water bath overnight, during which it will be gently shaken several times to help lysis. Or in a water bath at 55 ° C on a shaker for 3 h, vortex and shake at high speed once every hour. Make sure that the tail is completely lysed (the tail that is not cut may not be completely lysed, and the yield will be lower).

ð Add 1.8 μl RNase A (10 mg / ml) to the lysate, the final concentration of RNase A is 30 μg / ml, invert and mix, and incubate at 37 ° C for 15-30 m to remove residual RNA. Then cool at room temperature for at least 5 minutes or return to room temperature in an ice bath.
ð After adding 200 μl of protein precipitation solution to the lysate that has returned to room temperature, mix continuously at high speed on a vortex shaker for 25 sec. Some small protein clumps may be seen after mixing. Ice bath for 5 minutes.
Due to the small volume and weight of the sample, the shear force generated by vortexing and mixing will not cut and disrupt the genomic DNA. If you shake and mix by hand, don't shake it up and down vigorously.
The genomic DNA must be shaken and mixed with proper strength; otherwise, the genomic DNA will be cut; but the strength should not be too small. Make sure to mix thoroughly and break up the viscous lysate, otherwise the DNA cannot be separated from the protein precipitate, and it will be broken during centrifugation. Precipitate together with protein, causing DNA loss or reducing yield. In addition, insufficient mixing may also cause insufficient protein precipitation, and the final product may contaminate a larger amount of protein. Therefore, it is recommended to use a vortex oscillator.
ð Centrifuge at 13,000 rpm for 5 minutes. At this time, you should see protein deposits at the bottom of the tube, or you may see some protein deposits floating on the surface of the liquid.
ð Pour the supernatant into the filter column, centrifuge at 12,000 rpm for 20 sec, and collect the filtrate into a new 1.5 ml centrifuge tube.
ð Add an equal volume of room temperature isopropanol (approximately 600 μl) and mix by inversion 30 times or until cotton-like (filamentous) white DNA precipitate appears.
Note: When mixing upside down, the cotton-like (filamentous) DNA sometimes adheres to the lid or nozzle, and does not follow it down even if it is upside down. This will cause the operator to fail to see the precipitate and mistakenly believe that no DNA has been obtained. The solution is to omit step 9 and directly centrifuge at 12,000 rpm for 1 min, discard the supernatant, and then proceed to step 10. ð Place the centrifuge tube vertically to allow the white DNA pellet to sink to the bottom of the tube naturally, and then aspirate and discard most of the supernatant as much as possible, taking care not to aspirate the pellet.
If the cotton-like (filament) DNA precipitate has bubbles attached to it, it will float on the surface of the liquid and will not settle down. Be careful to avoid the precipitate and aspirate the supernatant.
ð Add 1ml 70% ethanol, rinse the DNA pellet upside down, and centrifuge at 12,000 rpm for 1 min. A white DNA pellet can be seen at the bottom of the tube. Discard the supernatant.
ð Add 1 ml 70% ethanol, invert several times to rinse the DNA pellet, centrifuge at 12,000 rpm for 1 min, discard the supernatant (be careful not to pour out the DNA pellet), and tap it on absorbent paper for a few times After inverting to control the residual ethanol, You can also use a pipette tip to carefully suck off the residual ethanol around the bottom of the tube and the wall of the tube, and air dry the precipitate for several minutes.
Be careful not to over-dry, otherwise the DNA is extremely insoluble; too much ethanol should not remain, otherwise the ethanol may inhibit downstream reactions such as enzyme digestion.

ð Add 100 μl DNA dissolving solution to rehydrate and dissolve the DNA pellet, flick the tube wall to mix, and incubate at 65 ° C for 30-60 min (not more than one hour). Flick the tube wall from time to time to help rehydrate the DNA. It can also be left at room temperature or 4 ° C overnight to rehydrate the DNA.
ð DNA can be stored at 2-8 ° C, if it is to be stored for a long time, it can be stored at -20 ° C.