Medium amount whole blood genomic DNA extraction kit (solution type)

Item No.: HRP405S 20 times
HRP405M 50 times

Kit Composition	Save	20Times	50Times
10x red blood cell lysate RSL	Room Temperature	40 ml	100 ml
Nuclear Lysate CL	Room Temperature	60 ml	150 ml
Protein precipitation solution	Room Temperature	20 ml	50 ml
DNA dissolving solution	Room Temperature	10 ml	20 ml

Storage matters:

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 to recover clarification. Do not shake it vigorously to avoid excessive foam formation.
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 adopts several rapid steps to extract genomic DNA according to the characteristics of whole blood. First, the red blood cell lysate is lysed to remove red blood cells that do not contain DNA. The nuclear lysate lyses white blood cells to release genomic DNA. Then the protein precipitation solution is selectively precipitated to remove proteins. Finally, the pure genomic DNA is precipitated with isopropanol and re-dissolved in the DNA lysis solution.

Features:

1. The red blood cell lysate formula selected from more than a dozen formulas, the lysis is fast and complete 2. No need to use toxic phenol and other reagents.
3. Fast and simple, a single sample operation can generally be completed within 30 minutes.
4. The results are stable and the yield is high (a typical yield of 3 ml whole blood can extract 50-150 μg), the typical ratio of OD260/OD280 is 1.7-1.9, and the length can reach 50Kb-150kb, which can be directly used for library construction and PCR , Southern-blot and various enzyme digestion reactions.

Precautions

a. All centrifugation steps are completed at room temperature, the speed can reach 2,500 x g, and the centrifuge is equipped with a 15 ml centrifuge tube rotor.
b. You need to bring your own isopropanol and 70% ethanol.
c. A typical yield of 3 ml of whole blood can extract 75-150 µg of genomic DNA (the number of white blood cells in different samples, especially disease samples, may vary greatly, so individual differences in yield may also be very large).
d. This kit is a solution type, which can easily scale up or down the whole blood volume per treatment (20 µl-10 ml). Please contact us for the operation manual for other treatment volumes e. This kit can be used in whole blood with a variety of anticoagulants, such as EDTA, citric acid, and heparin for anticoagulation. Among them, the leukocyte pellets of heparin anticoagulated blood are difficult to break up and resuspend, which affects the lysis effect. It is recommended to use non-heparin anticoagulants to collect blood samples.
f. For best results, it is best to use fresh blood specimens or specimens stored at 4°C for less than 3 days. Do not use specimens that have been repeatedly frozen and thawed more than 3 times, otherwise the yield will be severely reduced?

Operation steps: (please read the precautions before the experiment)
ð Pipette 9 ml ddH2O and 1 ml 10x red blood cell lysate into a 15 ml centrifuge tube.
ð After inverting the anticoagulated whole blood (return to room temperature before use), add 3 ml to the centrifuge tube containing the red blood cell lysate in the previous step, invert 6-8 times, and flip the tube wall upside down to ensure sufficient Mix well.
ð Leave it at room temperature for 10 minutes (during this time, it should be flipped upside down and mixed several times to help lyse the red blood cells).
ð Centrifuge at 2,500 xg for 2 min, discard the red supernatant, and carefully aspirate as much of the supernatant as possible (be careful not to aspirate the cell cluster at the bottom of the tube), leaving a complete white blood cell cluster at the bottom of the tube and about 50 µl residue Supernatant.
After centrifugation, you should see white white blood cell clusters at the bottom of the tube, and there may be some red blood cell fragments and white blood cell clusters together, but if you see most of the red cell clusters, it means that the red blood cell lysis is not sufficient and you should repeat step 1- 4 Operation, re-lyse red blood cells.
ð Vortex until the leukocyte cluster is fully resuspended and dispersed.
The resuspension and dispersion of white blood cells is very important for the next step of lysis. If the white blood cells are not broken up, adding the nucleus lysis solution will cause the white blood cells to not be fully lysed and form visible clumps.
ð Add 3 ml of nuclear lysis buffer to the resuspended white blood cells, pipette up and down to lyse the white blood cells, or vortex vigorously for 10 sec to help lyse the white blood cells.
ð Optional step, generally not required: Add RNase A (10 mg/ml) to the lysate to a final concentration of 30 µg/ml, invert 25 times to mix, incubate at 37°C for 15 minutes to remove residual RNA, then cool back to room temperature .
ð After adding 1 ml of protein precipitation solution, mix continuously at high speed on a vortex shaker for 25 sec. Some small protein clumps may be seen after mixing.

ð 2,500 x g (can be adjusted to increase the centrifugal force as needed) centrifugation for 5 min. At this time, you should see the dark brown protein deposits at the bottom of the tube, or you may see some protein deposits floating on the surface of the liquid.
ð Carefully pipette the supernatant (approximately 3 ml) into a new 15 ml centrifuge tube. When aspirating the supernatant, be careful not to aspirate the bottom of the tube and the protein precipitate floating on the surface of the liquid. If you accidentally transfer the protein pellet to a new centrifuge tube, you can centrifuge again for 2 minutes and then take the supernatant.
ð Add an equal volume of room temperature isopropanol (3 ml) and mix gently by inverting 30 times or until a cotton-like (filamentous) white DNA precipitate appears.
ð Centrifuge at 2,000 x g for 3 min. A white DNA pellet can be seen at the bottom of the tube. Discard the supernatant.
ð Add 3 ml of 70% ethanol, invert several times to rinse the DNA pellet, centrifuge at 2,000 xg for 1 min, pour out the supernatant (be careful not to pour out the DNA pellet), and then tap it on absorbent paper a few times to control the residue. Ethanol, you can also use a pipette tip to carefully suck the remaining ethanol around the bottom of the tube and the wall of the tube, and air dry the precipitate for a few 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 250 μl DNA dissolving solution to re-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.