Universal DNA Extraction Kit

(Silicone Membrane Centrifugal Column Method)

Cat. No./Spec.: N1211/24 preps; N1212/100 preps

Kit Contents

Component	N1211	N1212
Buffer ATL	10 ml	30 ml
Buffer AL	10 ml	30 ml
Buffer SW1	20 ml	60 ml
Buffer SW2	30 ml	110 ml
Proteinase K	0.6 ml	2.5 ml
Elution Buffer	10 ml	20 ml
DNA Spin Column	24 pcs	100 pcs
Collection Tube	24 pcs	100 pcs

Description

This product utilizes column purification technology, suitable for extracting high-purity DNA from animal tissues, processed animal-derived samples, cultured cells, exfoliated cells, blood, saliva, swabs, bloodstains, bacteria, and other samples. The purification principle involves lysing cells with cell lysis solution to release genomic DNA, which is then reversibly adsorbed by the silicone membrane column. After digestion with proteinase K and washing with wash solution to remove proteins, lipids, and polysaccharides, the genomic DNA is eluted with pure water to obtain the genomic DNA. The extraction process does not require the use of toxic phenol-chloroform extraction or time-consuming alcohol precipitation. The digestion process after extraction only takes 20 minutes. The obtained DNA can be directly used for PCR, quantitative PCR, Southern Blot, viral DNA detection, and second-generation sequencing experiments.

Storage

This product can be stored at room temperature (15~25˚C) for 18 months. Buffer ATL may precipitate at low temperatures, which can be dissolved by incubating at 55°C and mixing thoroughly. The product uses PET reagent bottles, which should not be subjected to high-temperature, high-pressure sterilization or direct incubation above 55°C.

Protocol

A. Solid Tissue Samples (not exceeding 50mg)

1. Cut the sample into small pieces and transfer to a 1.5ml centrifuge tube. Add 250μl Buffer ATL and 20μl Proteinase K Solution, incubate at 55˚C for 30~120 minutes or until the sample is completely digested.

• Correct tissue amount is essential for ideal results; too much sample can reduce yield and purity. Spleen and thymus are rich in DNA, do not exceed 10mg. Liver, kidney, lung, and other tissues should not exceed 20mg, while muscle and skin can be up to 50mg to increase DNA yield. The maximum amount for a mouse tail is 1.2cm, and for a rat tail is 0.6cm. For ethanol-preserved samples, rinse or air dry before proceeding.

• Cutting the tissue into smaller pieces can shorten digestion time. Liquid nitrogen grinding or glass homogenization can reduce digestion time. The digestion time depends on the sample type and homogenization effect. Generally, tissue samples need 0.5~2 hours, mouse tails need 2~4 hours, and overnight incubation at 55˚C has no negative impact.

• For processed animal-derived samples, such as cured meat, meat floss, leather, etc., take 30~100mg of the sample, mince or grind in an appropriate amount of PBS or physiological saline, centrifuge at 2,500 x g for 5~10 minutes to obtain cell sediment, and remove the supernatant containing grease or additives. Since the additives in processed animal-derived samples vary, they can be washed with PBS, physiological saline, ethanol, chloroform, or other reagents according to the actual situation, and then add 250~500μl ATL and 20μl Proteinase K Solution, and incubate overnight at 55˚C.

• For hair, feather, nail, etc., samples, add 10μl 1M DTT to assist digestion.

• To remove RNA: Add 5μl RNase Solution to the digestion solution, mix well, and place at room temperature for 10-15 minutes.

• To remove undigested impurities: If the digestion solution is not transparent or has particles, centrifuge at 13,000 x g for 3 minutes, and transfer the supernatant to a new centrifuge tube.

2. Add 250μl Buffer AL, vortex mix for 10 seconds, and incubate at 70°C for 10 minutes.

• If the digestion solution is not viscous, the incubation step can be omitted. Buffer AL and anhydrous ethanol can be premixed and added together to simplify the operation.

3. Add 250μl anhydrous ethanol, vortex mix for 15 seconds, and proceed to step 5.

• When processing enriched DNA samples (liver or spleen), the formation of precipitate upon the addition of anhydrous ethanol is a normal phenomenon.

B. Cell Samples (not exceeding 5 x 10^6)

1. Take an appropriate amount of cell culture fluid, amniotic fluid, urine, pleural effusion, etc., to a centrifuge tube, centrifuge at 2,000 x g for 10 minutes to collect cells. Carefully discard the culture fluid, leaving 100μl residual fluid and cell sediment, vortex or tap to loosen the cells.

• The amount of body fluid depends on the sample type, 5-15ml amniotic fluid, 10-50ml urine. Pleural effusion contains sputum, first use a 1% DTT solution to fully liquefy before centrifugation.

2. Add 150μl Buffer ATL and 20μl Proteinase K Solution to the sample, vortex mix. Incubate at 55°C for 10~30 minutes.

• To remove RNA: Add 5μl RNase Solution, mix well. Place at room temperature for 10-15 minutes.

3. Add 250μl Buffer AL to the sample, vortex for 5 seconds. Incubate at 70°C for 10 minutes.

• If the digestion solution is not viscous, the incubation step can be omitted. Buffer AL and anhydrous

ethanol can be premixed and added together to simplify the operation.

4. Add 250μl anhydrous ethanol to the sample, vortex for 5 seconds, and proceed to step 5.

C. Blood/Saliva and other liquid samples (250μl)

1. In a 1.5ml centrifuge tube, add 20μl Proteinase K Solution and 250μl of blood, plasma, cell suspension, saliva, swab soaking solution, etc.

• Nucleated red blood cells: The red blood cells of non-mammalian animal blood, such as birds/fish, are nucleated and contain a rich amount of DNA. In a 1.5ml centrifuge tube, add 10~50μl of anticoagulated blood, dilute with Buffer PBS or physiological saline to 250 μl.

• Clotted blood: Homogenize the blood clot into a uniform liquid using an electric/glass homogenizer or other methods.

• Blood and body fluids: For concentrated samples such as human blood yellow layer/lymphocytes, do not exceed 1ml of whole blood before concentration, too many lymphocytes can make the lysis solution too viscous and affect extraction. For other mammalian blood, it is recommended to use 150μl first, and then make up to 250 μl with sterile water. If the blood or body fluid sample is less than 250 μl, make up to 250 μl with sterile water.

2. Add 250μl Buffer AL, invert 5-10 times, vortex for 10 seconds. Incubate at 70°C for 10 minutes.

• Thorough mixing is very important. For viscous samples, invert several times before vortexing to achieve a whirlpool effect. Set the temperature of the metal bath to 800-1500rpm. When incubating in a water bath, invert and flip twice during the incubation to accelerate digestion.

3. Add 250μl anhydrous ethanol, vortex for 10 seconds. Proceed to step 5.

D. Bloodstains, Semen Stains Samples

1. Use a punch to cut out 3-5 pieces of 3mm diameter circles from dry blood slides, transfer to a 2.0ml centrifuge tube. Add 300μl Buffer ATL and 20μl Proteinase K Solution, incubate at 55℃ with oscillation (1200~1400rpm) for 60 minutes.

• For processing more blood slides, increase the amount of Buffer ATL and Proteinase K Solution accordingly, thorough oscillation is key to yield. For semen stain samples, add 10μl 1M DTT to assist digestion.

2. Add 200μl Buffer AL to the sample, incubate at 70°C with oscillation (1200~1400rpm) for 15 minutes.

3. Centrifuge at 13,000 x g for 1 minute to collect the droplets on the tube wall. Transfer 450~500μl of the digestion solution to a 1.5ml centrifuge tube.

4. Add 250μl anhydrous ethanol, vortex for 5 seconds. Proceed to step 5.

E: Bacteria (not exceeding 2×10^9 bacteria)

1. Take an appropriate amount of bacterial culture fluid, urine, homogenate, swab soaking solution, food fermentation liquid, etc., to a centrifuge tube, centrifuge at 10,000 x g for 3 minutes to collect bacteria, carefully discard the supernatant.

• Gram-negative bacteria: Resuspend the bacterial pellet with sterile water or TE, or directly take 300μl of bacterial fluid or liquid samples, proceed to step 2.

• Gram-positive bacteria: Resuspend the bacterial pellet with 300μl Buffer TE and 10μl Lysozyme (100mg/ml), vortex. Place at room temperature for 15 minutes.

2. Add 200μl Buffer AL and 20μl Proteinase K Solution, vortex for 10 seconds, incubate at 65°C for 20 minutes.

• If the digestion solution is not transparent or has obvious particles, centrifuge at 10,000 x g for 1 minute to remove undigested impurities.

• To remove RNA: Add 5μl RNase Solution, mix well. Place at room temperature for 10-15 minutes.

3. Add 250μl anhydrous ethanol, vortex for 5 seconds. Proceed to step 5.

F: Yeast (not exceeding 1 × 10^7)

1. Take an appropriate amount of yeast culture fluid to a centrifuge tube, centrifuge at 10,000 x g for 1 minute to collect cells, carefully discard the supernatant. Add 300μl Buffer ATL and 300mg of acidic glass beads (0.4-0.5mm), vortex at high speed for 10 minutes.

• To remove RNA: Add 5μl RNase Solution, mix well. Place at room temperature for 10-15 minutes.

2. Centrifuge at 10,000 x g for 1 minute, transfer 250μl of the homogenate to a new centrifuge tube.

3. Add 250μl Buffer AL and 20μl Proteinase K Solution to the sample, vortex for 5 seconds. Incubate at 70°C for 10 minutes.

4. Add 250μl anhydrous ethanol to the lysis solution, vortex for 5 seconds. Proceed to step 5.

Column Purification

5. Place the purification column in a 2ml collection tube. Transfer the mixture (including precipitate) to the column. Centrifuge at 12,000 x g for 1 minute.

• If clogging occurs during this step, increase the centrifugal speed to 15,000 x g. Adjust the sample amount before the next experiment or add an additional centrifugation step before adding ethanol to remove undigested samples.

6. Discard the filtrate, place the column back in the collection tube. Add 500μl Buffer SW1. Centrifuge at 12,000 x g for 1 minute.

7. Discard the filtrate, place the column back in the collection tube. Add 750μl Buffer SW2 to the column. Centrifuge at 12,000 x g for 1 minute.

• For samples with low nucleic acid content, if the obtained DNA concentration is below 50ng/μl, it is recommended to divide Buffer SW2 into two washes, each with 500μl, to stabilize the A260/230 ratio.

8. Discard the filtrate, place the column back in the collection tube. Centrifuge at 12,000 x g for 2 minutes.

9. Place the column in a new 1.5ml centrifuge tube. Apply 50~100μl Elution Buffer to the purification column membrane, let it stand at room temperature for 3 minutes, centrifuge at 12,000 x g for 1 minute.

10. Apply 50~100μl Elution Buffer to the purification column again, let it stand at room temperature for 3 minutes. Centrifuge at 12,000 x g for 1 minute.

11. Discard the DNA binding column, store the DNA at 2~8°C, and for long-term storage, store at -20°C.

This product is for scientific research use only.