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EasySC Universal DNA Purification Kit

Catalog Number: D135-1, D135-2

Table 1. Kit Components and Storage

Kit Component	D135-1 (50 preps)	D135-2 (200 preps)	Storage	Stability
Buffer ATL	15 mL	60 mL	RT	The product is stable for one year when stored as directed.
Buffer DL	15 mL	60 mL	RT	
Buffer AW1*	15 mL	53 mL	RT	
Buffer AW2*	15 mL	2×25 mL	RT	
Buffer AE	15 mL	30 mL	RT	
Proteinase K (20 mg/mL)	1 mL	4×1 mL	-20℃	
Mini Column	50	200	RT	
2 mL Collection Tube	50	200	RT	

***** Prior to use, add absolute ethanol to **Buffer AW1, Buffer AW2** according to the bottle label.

Product Description

EasySC Universal DNA Purification Kit is designed for rapid purification of genomic DNA from a variety of sample sources including fresh or frozen animal tissues and cells, blood, serum, plasma, saliva, swab, sperm, blood spot. The DNA purification process is simplified with Spin Column technology into four quick "lyse-bind-wash-elute" steps and can be accomplished in less than 20 minutes post-lysis. This convenient spin-column format avoids time-consuming steps like alcohol precipitation, use of toxic compounds such as phenol and chloroform and allows for multiple samples to be processed in parallel. DNA purified using this kit is ready for most downstream applications such as PCR, sequencing, genotyping, southern blot analysis and restriction enzyme digestion.

Features

- ❖ Fast – DNA purification process in less than 20 min post-lysis.
- ❖ Safe – No Phenol/chloroform extractions.
- ❖ High-quality – DNA is suitable for a variety of downstream applications.

Things to do before starting

- Buffer DL may form precipitates upon storage. If necessary, warm to 50°C to redissolve.
- Buffer AW1 and Buffer AW2 are supplied as concentrates. Before using for the first time, add the appropriate amount of ethanol (96-100%) as indicated on the bottle to obtain a working solution.
- Preheat a water bath or heating block to 55°C and 70°C.
- Prepare RNase A solution (10 mg/mL).
- Prepare 1 M DTT solution.

Protocol: Isolation Genomic DNA from Tissue Sample

1. Weigh ~25 mg tissue (or <10 mg liver, lung, or spleen tissue), and cut the tissue into small pieces, then transfer it into a 1.5 mL microcentrifuge tube.

   Note: Using too much tissue sample may reduce yield and purity. Spleen, liver, and kidney tissues are rich in DNA, and should be used no more than 10 mg. Muscle and skin tissues can be used at 30 mg. Liquid nitrogen grinding, mechanical homogenizer, or glass homogenizer can be used to homogenize tissue sample to reduce digestion time.

2. Add 230 µL Buffer ATL and 20 µL Proteinase K solution. Vortex to mix thoroughly.

3. Incubate at 55°C for 1-3 hr or overnight. Vortex briefly every 20-30 min during incubation, or incubate with shaking.

   Note: Lysis time depends on the amount and type of tissue used. The average time is 0.5-3 hr, and mouse tails may take 6-8 hr. Lysis can be proceed overnight.

4. (*Optional*) If RNA-free genomic DNA is required, add 20 µL RNase A (10 mg/mL). Mix by vortexing, and incubate at RT for 10 min.

5. Centrifuge at 12,000 x g for 3 min. Transfer the supernatant to a new 1.5 mL microcentrifuge tube.

6. Add 250 µL Buffer DL. Vortex to mix thoroughly. Incubate at 70°C for 10 min.

   Note: Buffer DL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve. A precipitate may form upon the addition of Buffer DL. This precipitate does not interfere with the DNA recovery.

7. Add 250 µL 100% ethanol. Vortex to mix thoroughly.

   Note: A precipitate may form upon the addition of ethanol when processing liver or spleen tissue. Pipette up and down 5-10 times to break up the sediment.

8. Connect a DNA Mini Column to a 2 mL Collection Tube. Transfer the entire sample from step 7 into the DNA Mini column including any precipitates that may have formed. Centrifuge at 12,000 x g for 1 min.

9. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW1 to the DNA Mini Column, then centrifuge at 12,000 x g for 1 min.

   Note: Buffer AW1 must be diluted with absolute ethanol according to the bottle label before use.

10. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW2 to the DNA Mini Column, then centrifuge at 12,000 x g for 1 min.

    Note: Buffer AW2 must be diluted with absolute ethanol according to the bottle label before use.

11. Repeat step 10 for a second wash step.

12. Discard the filtrate and reuse the collection tube. Centrifuge the empty DNA Mini Column at 12,000 x g for 3 min.

    Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.

13. Transfer the DNA Mini Column into a clean 1.5 mL microcentrifuge tube. Add 30-100 µL Buffer AE preheated to 70°C. Close the lid and incubate at RT for 3 min, then centrifuge at 12,000 x g for 1 min.

    Note: To improve the yield, repeat this step for a second elution step.

14. Discard the column and store the DNA at -20ºC.

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Protocol: Isolation Genomic DNA from Blood Sample

1. Add 20 µL Proteinase K solution into a 1.5 mL microcentrifuge tube.
2. Transfer 250 µL fresh or frozen anticoagulated whole blood to the 1.5 mL microcentrifuge tube containing Proteinase K. Vortex for 5 seconds.

   Note: When processing coagulated blood samples, homogenize the samples with a mechanical or glass homogenizer to fully liquefy them before extracting. Since red blood cells of non-mammalian animals such as birds and fish are nucleated, their DNA content is extremely rich, and the kit can only process 5-20 µL of blood at a time.
3. Add 250 µL Buffer DL to the sample. Vortex thoroughly for 15 seconds, and then incubate at 70°C for 10 min. Vortex briefly once during incubation.

   Note: Buffer DL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve.
4. (*Optional*) If RNA-free genomic DNA is required, add 20 µL RNase A (10 mg/mL). Mix by vortexing, and incubate at RT for 10 min.
5. Add 250 µL isopropanol to the sample, and vortex for 15 seconds. Centrifuge briefly to collect any drops from the inside of the lid.
6. Insert a DNA Mini Column into a 2 mL Collection Tube. Transfer the sample from Step 5 to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min at RT.
7. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW1 to the DNA Mini Column, invert and mix once, then centrifuge at 10,000 x g for 1 min.

   Note: Buffer AW1 must be diluted with absolute ethanol according to the bottle label before use.
8. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW2 to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min.

   Note: Buffer AW2 must be diluted with absolute ethanol according to the bottle label before use.
9. Repeat step 8 for a second wash step.
10. Discard the filtrate and reuse the collection tube. Centrifuge the empty DNA Mini Column at 12,000 x g for 3 min.

    Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.
11. Transfer the DNA Mini Column into a clean 1.5 mL microcentrifuge tube, add 30-100 µL Buffer AE preheated to 70ºC. Let sit at RT for 5 min, then centrifuge at 10,000 x g for 1 min.

    Note: To improve the yield, repeat this step for a second elution step.
12. Discard the column and store the DNA at -20ºC.

Protocol: Isolation Genomic DNA from Serum, Plasma, or Saliva Sample

1. Add 20 µL Proteinase K solution into a 2 mL microcentrifuge tube.
2. Transfer 500 µL serum, plasma, or saliva sample to the 2 mL microcentrifuge tube containing Proteinase K. Vortex for 5 seconds.
3. Add 500 µL Buffer DL to the sample. Vortex thoroughly for 15 seconds, and then incubate at 70°C for 10 min. Vortex briefly once during incubation.

   Note: Buffer DL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve.
4. (*Optional*) If RNA-free genomic DNA is required, add 20 µL RNase A (10 mg/mL). Mix by vortexing, and incubate at RT for 10 min.
5. Add 500 µL isopropanol to the sample, and vortex for 15 seconds. Centrifuge briefly to collect any drops from the inside of the lid.
6. Insert a DNA Mini Column into a 2 mL Collection Tube. Transfer 600 µL mixture from step 5 to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min at RT.
7. Discard the filtrate and reuse the collection tube. Transfer remaining sample from step 5 to the DNA Mini Column. Centrifuge at 10,000 x g for 1 min.
8. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW1 to the DNA Mini Column, invert and mix once, then centrifuge at 10,000 x g for 1 min.

   Note: Buffer AW1 must be diluted with absolute ethanol according to the bottle label before use.
9. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW2 to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min.

   Note: Buffer AW2 must be diluted with absolute ethanol according to the bottle label before use.
10. Repeat step 9 for a second wash step.
11. Discard the filtrate and reuse the collection tube. Centrifuge the empty DNA Mini Column at 12,000 x g for 3 min.

    Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.
12. Transfer the DNA Mini Column into a clean 1.5 mL microcentrifuge tube, add 30-100 µL Buffer AE preheated to 70ºC. Let sit at RT for 5 min, then centrifuge at 10,000 x g for 1 min.

    Note: To improve the yield, repeat this step for a second elution step.
13. Discard the column and store the DNA at -20ºC.

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Protocol: Isolation Genomic DNA from Cultured Cells

1. Prepare the cell suspension using one of the following methods:
   - Frozen cell samples should be thawed before starting this protocol. Pellet the cells by centrifugation. Wash the cells with cold PBS (4°C). Resuspend cells in 230 µL PBS. Proceed to Step 2.
   - For cells grown in suspension, pellet 5 x 10^6 by spinning at 1,200 x g in a centrifuge tube. Aspirate and discard the supernatant, and wash the cells once with cold PBS (4°C). Resuspend cells in 230 µL PBS. Proceed to Step 2.
   - For cells grown in a monolayer, harvest the cell by either using a trypsin treatment or by scraping with a rubber policeman. Wash cells twice with cold PBS (4°C). Resuspend the cells in 230 µL PBS. Proceed to Step 2.
2. Add 20 µL Proteinase K solution. Vortex for 15 seconds. If RNA-free genomic DNA is required, add 20 µL RNase A (10 mg/mL).
3. Add 250 µL Buffer DL to the sample. Vortex thoroughly for 15 seconds, and then incubate at 65°C for 15 min. Vortex briefly once during incubation.

   Note: Buffer DL may be precipitated during storage, if happen, heat it at 50°C to dissolve.
4. Add 250 µL isopropanol to the sample, and vortex for 15 seconds. Centrifuge briefly to collect any drops from the inside of the lid.
5. Insert a DNA Mini Column into a 2 mL Collection Tube. Transfer the sample from step 4 to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min at RT.
6. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW1 to the DNA Mini Column, invert and mix once, then centrifuge at 10,000 x g for 1 min.

   Note: Buffer AW1 must be diluted with absolute ethanol according to the bottle label before use.
7. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW2 to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min.

   Note: Buffer AW2 must be diluted with absolute ethanol according to the bottle label before use.
8. Repeat step 7 for a second wash step.
9. Discard the filtrate and reuse the collection tube. Centrifuge the empty DNA Mini Column at 12,000 x g for 3 min.

   Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.
10. Transfer the DNA Mini Column into a clean 1.5 mL microcentrifuge tube, add 30-100 µL Buffer AE preheated to 70ºC. Let sit at RT for 5 min, then centrifuge at 10,000 x g for 1 min.

    Note: To improve the yield, repeat this step for a second elution step.
11. Discard the column and store the DNA at -20ºC.

Protocol: Isolation Genomic DNA from Swab Sample

1. Place swab sample in a 2 mL microcentrifuge tube.
2. Add 500 µL Buffer ATL and 20 µL Proteinase K solution. Vortex to mix thoroughly.
3. Incubate at 55°C for 1 hr. Vortex briefly every 10 min during incubation, or incubate with shaking.
4. Remove the swab by pressing it against the walls of the tube to squeeze out most of the liquid. Then, centrifuge at 12,000 x g for 3 min. Transfer the supernatant to a new 2 mL microcentrifuge tube.
5. Add 500 µL Buffer DL. Vortex to mix thoroughly. Incubate at 70°C for 10 min.

   Note: Buffer DL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve.
6. Add 500 µL isopropanol to the sample, and vortex for 15 seconds. Centrifuge briefly to collect any drops from the inside of the lid.
7. Connect a DNA Mini Column to a 2 mL Collection Tube. Transfer 600 µL mixture from step 6 to the DNA Mini Column, then centrifuge at 10,000 x g for 1 min at RT.
8. Discard the filtrate and reuse the collection tube. Transfer remaining sample from step 6 to the DNA Mini Column. Centrifuge at 10,000 x g for 1 min.
9. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW1 to the DNA Mini Column, invert and mix once, then centrifuge at 12,000 x g for 1 min.

   Note: Buffer AW1 must be diluted with absolute ethanol according to the bottle label before use.
10. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW2 to the DNA Mini Column, then centrifuge at 12,000 x g for 1 min.

    Note: Buffer AW2 must be diluted with absolute ethanol according to the bottle label before use.
11. Repeat step 10 for a second wash step.
12. Discard the filtrate and reuse the collection tube. Centrifuge the empty DNA Mini Column at 12,000 x g for 3 min.

    Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.
13. Transfer the DNA Mini Column into a clean 1.5 mL microcentrifuge tube. Add 30-100 µL Buffer AE preheated to 70°C. Close the lid and incubate at RT for 3 min, then centrifuge at 12,000 x g for 1 min.

    Note: To improve the yield, repeat this step for a second elution step.
14. Discard the column and store the DNA at -20ºC.

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Protocol: Isolation Genomic DNA from Blood or Sperm Spot

1. Use a punch to cut out 3-5 blood-bearing discs with a diameter of ~3 mm from the dried blood slices. Transfer it to a 2 mL microcentrifuge tube.
2. Add 230 µL Buffer ATL and 20 µL Proteinase K solution. Vortex to mix thoroughly. When processing sperm spot sample, add additional 10 µL of 1 M DTT.
3. Incubate at 55°C for 30-60 min. Vortex briefly every 10 min during incubation, or incubate with shaking.
4. Add 250 µL Buffer DL. Vortex to mix thoroughly. Incubate at 70°C for 15 min.

   Note: Buffer DL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve.
5. Centrifuge at 10,000 x g for 1 min. Transfer the supernatant to a new 1.5 mL microcentrifuge tube.
6. Add 250 µL 100% ethanol. Vortex to mix thoroughly.
7. Connect a DNA Mini Column to a 2 mL Collection Tube. Transfer the entire sample from step 6 into the DNA Mini column. Centrifuge at 12,000 x g for 1 min.
8. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW1 to the DNA Mini Column, then centrifuge at 12,000 x g for 1 min.

   Note: Buffer AW1 must be diluted with absolute ethanol according to the bottle label before use.
9. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW2 to the DNA Mini Column, then centrifuge at 12,000 x g for 1 min.

   Note: Buffer AW2 must be diluted with absolute ethanol according to the bottle label before use.
10. Repeat step 9 for a second wash step.
11. Discard the filtrate and reuse the collection tube. Centrifuge the empty DNA Mini Column at 12,000 x g for 3 min.

    Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.
12. Transfer the DNA Mini Column into a clean 1.5 mL microcentrifuge tube. Add 30-100 µL Buffer AE preheated to 70°C. Close the lid and incubate at RT for 3 min, then centrifuge at 12,000 x g for 1 min.

    Note: To improve the yield, repeat this step for a second elution step.
13. Discard the column and store the DNA at -20ºC.

Protocol: Isolation Genomic DNA from Hair or Nail Sample

1. Cut hair or nail sample into small pieces, and transfer it into a 1.5 mL microcentrifuge tube.
2. Add 230 µL Buffer ATL, 20 µL Proteinase K solution, and 10 µL DTT (1 M). Vortex to mix thoroughly.
3. Incubate at 55°C for 3 hr. Vortex briefly every 20-30 min during incubation, or incubate with shaking.
4. Add 250 µL Buffer DL. Vortex to mix thoroughly. Incubate at 70°C for 10 min.

   Note: Buffer DL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve.
5. Centrifuge at 10,000 x g for 3 min. Transfer the supernatant to a new 1.5 mL microcentrifuge tube.
6. Add 250 µL 100% ethanol. Vortex to mix thoroughly.
7. Connect a DNA Mini Column to a 2 mL Collection Tube. Transfer the entire sample from step 6 into the DNA Mini column. Centrifuge at 12,000 x g for 1 min.
8. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW1 to the DNA Mini Column, then centrifuge at 12,000 x g for 1 min.

   Note: Buffer AW1 must be diluted with absolute ethanol according to the bottle label before use.
9. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW2 to the DNA Mini Column, then centrifuge at 12,000 x g for 1 min.

   Note: Buffer AW2 must be diluted with absolute ethanol according to the bottle label before use.
10. Repeat step 9 for a second wash step.
11. Discard the filtrate and reuse the collection tube. Centrifuge the empty DNA Mini Column at 12,000 x g for 3 min.

    Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.
12. Transfer the DNA Mini Column into a clean 1.5 mL microcentrifuge tube. Add 30-100 µL Buffer AE preheated to 70°C. Close the lid and incubate at RT for 3 min, then centrifuge at 12,000 x g for 1 min.

    Note: To improve the yield, repeat this step for a second elution step.
13. Discard the column and store the DNA at -20ºC.

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Protocol: Isolation Genomic DNA from Sperm Sample

1. Vortex sperm sample, and transfer 150 µL sample into a 1.5 mL microcentrifuge tube.
2. Add 100 µL Buffer ATL, 20 µL Proteinase K solution, and 10 µL DTT (1 M). Vortex to mix thoroughly.
3. Incubate at 55°C for 30-60 min. Vortex briefly every 10 min during incubation, or incubate with shaking.
4. Add 250 µL Buffer DL. Vortex to mix thoroughly. Incubate at 70°C for 10 min.

   Note: Buffer DL may be precipitated during storage, if happen, heat it at 50^{o}C to dissolve.
5. Add 250 µL 100% ethanol. Vortex to mix thoroughly.
6. Connect a DNA Mini Column to a 2 mL Collection Tube. Transfer the entire sample from step 5 into the DNA Mini column. Centrifuge at 12,000 x g for 1 min.
7. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW1 to the DNA Mini Column, then centrifuge at 12,000 x g for 1 min.

   Note: Buffer AW1 must be diluted with absolute ethanol according to the bottle label before use.
8. Discard the filtrate and reuse the collection tube. Add 600 µL of Buffer AW2 to the DNA Mini Column, then centrifuge at 12,000 x g for 1 min.

   Note: Buffer AW2 must be diluted with absolute ethanol according to the bottle label before use.
9. Repeat step 8 for a second wash step.
10. Discard the filtrate and reuse the collection tube. Centrifuge the empty DNA Mini Column at 12,000 x g for 3 min.

    Note: This step is critical for removing of trace ethanol that may interfere with downstream applications.
11. Transfer the DNA Mini Column into a clean 1.5 mL microcentrifuge tube. Add 30-100 µL Buffer AE preheated to 70°C. Close the lid and incubate at RT for 3 min, then centrifuge at 12,000 x g for 1 min.

    Note: To improve the yield, repeat this step for a second elution step.
12. Discard the column and store the DNA at -20ºC.