

Micro Sample DNA Fast Extraction Kit

(Spin-column)

The kit provides a fast way for the isolating of high-purity DNA from micro samples

for laboratory research use only.

DP3301 20 preps

DP3302 50 preps

Kit Content, Storage, and Stability

Content	Storage	20 preps	50 preps
Buffer A	RT	6 ml	15 ml
Buffer B	RT	6 ml	15 ml
Buffer C	RT	6 ml	15 ml
Buffer D	RT	10 ml	25 ml
Buffer EB	RT	6 ml	15 ml
Carrier	-20°C	35 µl	80 µl
Buffer WB	RT	3 ml	8 ml
	Add ration ethanol before use.		
Isopropanol	RT	3 ml	8 ml
Proteinase K (20mg/ml)	-20°C	8 mg	20 mg
Spin-column AC	RT	20	50
Collection Tube (2ml)	RT	20	50

All reagents are stable for 12 months at RT.

Store carrier at -20°C when dissolved.

Notes

Please add 100% ethanol to Buffer WB before starting, vortex adequately, then ticking on the block, avoid multi-adding!

Buffer C or D may precipitate under low temperature, then put incubation 37°C for a moment until clear, then cool to RT for use.

Proteinase K is provided as freeze-dried powder for activity and transportation. Please dissolve it in 1 ml sterile water after transient centrifuging when arrival. Because frozen and melt repeatedly may affect enzyme activity, please store in per dose under -20°C

Prepare 100% ethanol and 1M DTT (for isolation DNA from hair).

The maximum volume of Spin-column AC is 900 µl; the minimum elution volume is 15 µl. For samples, the maximum volume of blood is 100 µl and maximum weight for tissue is 10 mg.

Carrier is provided in this kit to precipitate micro DNA. Carrier is not nucleic acid, does not affect downstream experiments.

Principle

The kit applies the unique binding buffer/Proteinase K to rapidly lyse cells and inactivate cellular nucleases, then DNA selectively absorbs on silicified membrane in high salt solution. Take a series of elution-centrifugation steps to remove cellular metabolite and proteins. Finally low salt elution wash away purified genome DNA from silicified membrane. Carrier has been used in this kit for precipitate micro DNA. This kit can be used to isolate DNA from small scale blood and tissue, plasma, serum, bloodstain, hair follicle and other small scale samples.

Procedure

Please check ethanol has been added to Buffer WB before starting.

Protocol 1: Isolate genomic DNA from micro blood

1. Take 1-100 μ l fresh, frozen or anticoagulant blood into a 1.5 ml microtube. If the volume of all of whole blood is less than 100 μ l, please make it up to 100 μ l with Buffer A.
2. Add 10 μ l Proteinase K (20mg/ml), mix thoroughly.
3. Add 5 μ l RNase A (100mg/ml), vortex for 5 sec, incubate at RT for 5 min.
4. Add 100 μ l Buffer C (Add carrier 1 μ l per 10 μ l sample), then overturn to mix thoroughly, incubate at 56°C for 10 min.
5. If Buffer C forms white precipitate, it will be clear at 56°C when lyse cells completely. Or the precipitate will affect DNA yield and purity.
6. Add 50 μ l isopropanol, overturn to mix thoroughly, incubate at RT for 3 min.
7. Transfer whole solution to a Spin-column AC (in a Collection Tube), centrifuge at 12,000 rpm for 30 sec, discard filtrate and put the Spin-column AC back to Collection Tube.
8. Add 500 μ l Buffer D, centrifuge at 12,000 rpm for 30 sec, discard filtrate and put the Spin-column AC back to Collection Tube.
9. Add 500 μ l Buffer WB (check ethanol added!), centrifuge at 12,000 rpm for 45 sec, discard filtrate.
10. Transfer the Spin-column AC to a clear 1.5ml microcentrifuge tube, add 15-50 μ l Buffer EB, let it stay at RT for 2-5min, then centrifuge at 12,000 rpm for 2 min.
11. Buffer EB has not be less than 15 μ l, as low elution volume affects elution efficiency and final DNA yield.

Please add the elution of first time back to Spin-column AC and centrifuge again to obtain more DNA. pH of Buffer EB can affect elution efficiency. Please make sure pH is around 7.0-8.5 if elute DNA with water. pH less than 7.0 decreases elution efficiency.

Please keep DNA at -20°C.

Protocol 2: Isolate DNA from serum/plasma

1. Take 200 μ l liquid samples (serum, plasma, etc) to a 1.5 microtube. If the volume of sample is less than 200 μ l, please make it up to 200 μ l with Buffer A.
2. Add 200 μ l Buffer C, vortex 15 sec and mix thoroughly. Add 20 μ l Proteinase K (20mg/ml) mix thoroughly and incubate at 72°C for 10min or until it's clear.
3. Cool down to RT, add 100 μ l isopropanol, vortex and mix thoroughly, precipitate may form at this step.
4. Transfer all above solution to a Spin-column AC (in a Collection Tube), centrifuge at 10,000 rpm for 30 sec, then discard filtrate.

5. Add 500 µl Buffer D, centrifuge at 12,000 rpm for 30 sec, discard filtrate and put the Spin-column AC back to Collection Tube.
6. Add 500 µl Buffer WB (check ethanol added!), centrifuge at 12,000 rpm for 45 sec, discard filtrate.
7. Transfer the Spin-column AC to a clear 1.5 ml microcentrifuge tube, add 15 - 50 µl Buffer EB, let it stay at RT for 2-5 min, then centrifuge at 12,000 rpm for 2 min.
8. Buffer EB should be not less than 15 ul. Low elution volume affects elution efficiency and final DNA yield.
9. Please add the elution of first time back to Spin-column AC and centrifuge again to obtain more DNA.

DNA can be stored at 2-8 °C; Please keep DNA at -20 °C for long-term storage.

Protocol 3: Isolate DNA from bloodstain

1. Take 3 pieces 3×3 mm of samples into a 1.5 ml microcentrifuge.
2. Add 180 µl Buffer A. Add 20 µl Proteinase K (20mg/ml), mix thoroughly, incubate at 56°C for 1 h and overturn to mix every 10 min.
3. Add 200 µl Buffer C and 1ul Carrier, mix gently and incubate at 70°C for 10 min. If Buffer C forms white precipitate, it will be clear at 70°C when lyse cells completely. If not, the precipitate will affect DNA yield and purity.
4. Add 100 µl isopropanol, vortex and mix thoroughly; incubate at RT for 5 min.
5. Transfer all above mixture to a Spin-column AC (in a Collection Tube), centrifuge at 12,000 rpm for 30 sec, discard filtrate.
6. Add 500 µl Buffer D, centrifuge at 12,000 rpm for 30 sec, discard filtrate and put the Spin-column AC back to Collection Tube.
7. Add 500 µl Buffer WB (check ethanol added!), centrifuge at 12,000 rpm for 45 sec, discard filtrate.
8. Transfer the Spin-column AC to a clear 1.5 ml microcentrifuge tube, add 15 - 50 µl Buffer EB, let it stay at RT for 2-5 min, then centrifuge at 12,000 rpm for 2 min.
9. Buffer EB with volume less than 15 ul can affect elution efficiency and final DNA yield.

Please add the elution of first time back to Spin-column AC, and centrifuge again to obtain more DNA. pH of Buffer EB can affect elution efficiency. Please make sure pH is around 7.0-8.5 if elute DNA with water (adjust pH with NaOH). pH less than 7.0 decreases elution efficiency.

Please keep DNA at -20 °C.

Protocol 4: Isolate DNA from mouthwash

1. Add 10-20 ml mouthwash to a sterile 50 ml tube, centrifuge at 1,800 g for 5 min and discard the supernatant.
2. Add 200 µl Buffer A to resuspend pellet, transfer solution to a clean 1.5 ml microcentrifuge. Add 20µl Proteinase K (20mg/ml), mix thoroughly, incubate at 56°C for 1h and overturn to mix every 10 min.
3. Add 200 µl Buffer C and 1 ul Carrier, mix gently and incubate at 70°C for 10min.
4. If Buffer C forms white precipitate, it will be clear at 70°C when lyse cells completely. If not, the precipitate will affect DNA yield and purity.
5. Add 100 µl isopropanol, vortex and mix thoroughly; incubate at RT for 5 min.

6. Transfer all above mixture to a Spin-column AC (in a Collection Tube), centrifuge at 12,000 rpm for 30 sec, discard filtrate.
7. Add 500 µl Buffer D, centrifuge at 12,000 rpm for 30 sec, discard filtrate and put the Spin-column AC back to Collection Tube.
8. Add 500 µl Buffer WB (check ethanol added!), centrifuge at 12,000 rpm for 45 sec, discard filtrate.
9. Transfer the Spin-column AC to a clear 1.5ml microcentrifuge tube, add 15 - 50µl Buffer EB, let it stay at RT for 2-5 min, then centrifuge at 12,000 rpm for 2 min.

Buffer EB with volume less than 15µl can affect elution efficiency and final DNA yield. Please add the elution of first time back to Spin-column AC, and centrifuge again to obtain more DNA. pH of Buffer EB can affect elution efficiency. Please make sure pH is around 7.0-8.5 if elute DNA with water (adjust pH with NaOH). pH less than 7.0 decreases elution efficiency.

Please keep DNA at -20 °C.

Protocol 5: Isolate DNA from Hair Follicles

Please prepare 1M DTT before starting.

1. Add 250 µl Buffer, 20 µl Proteinase K (20mg/ml) and 20 µl 1M DTT to a 1.5 ml microcentrifuge, mix thoroughly. Take 1 cm hair root with hair follicle into mixture, vortex for 10 sec.
2. Incubate at 56°C for 60 min until sample lysis completely, vortex for 10 sec every 20 min.
3. Lysis duration depends on samples. Usually, 1 hour is long enough for hair follicles lysis. Lysis overnight could not affect DNA isolation. Take the supernatant for next steps for samples that cannot be lysed thoroughly.
4. Add 300 µl Buffer C and 1 µl Carrier, mix gently and incubate at 56°C for 10 min.
5. Add 300 µl 100% ethanol, vortex and mix thoroughly.
6. Transfer all above mixture to a Spin-column AC (in a Collection Tube), centrifuge at 12,000 rpm for 30 sec, discard filtrate.
7. Add 500µl Buffer D, centrifuge at 12,000 rpm for 30 sec, discard filtrate and put the Spin-column AC back to Collection Tube.
8. Add 500 µl Buffer WB (check ethanol added!), centrifuge at 12,000 rpm for 45 sec, discard filtrate.
9. Transfer the Spin-column AC to a clear 1.5ml microcentrifuge tube, add 15 - 50µl Buffer EB, let it stay at RT for 2-5 min, then centrifuge at 12,000 rpm for 2min.

Buffer EB with volume less than 15µl can affect elution efficiency and final DNA yield. Please add the elution of first time back to Spin-column AC and centrifuge again to obtain more DNA. pH of Buffer EB can affect elution efficiency. Make sure pH is around 7.0-8.5 if elute DNA with water (adjust pH with NaOH). pH less than 7.0 will decrease elution efficiency.

Please keep DNA at -20 °C.

Protocol 6: Isolate DNA from micro tissues

1. Take less than 10 mg tissue powder to a clean 1.5 microcentrifuge tube with 100 µl Buffer B, mix by pipetting using big caliber tips.
2. Add 10 µl Proteinase K (20mg/ml), turnover and mix thoroughly. Incubate at 55°C for 60 min or till thorough digestion and mix gently to help digest.
3. Add 100 µl buffer C, overturn to mix thoroughly, and incubate at 70°C for 10 min.

4. Cool down to RT, add 50 µl isopropanol, then overturn to mix thoroughly.
5. Transfer the mixture into a Spin-column AC (place the Spin -column AC to Collection Tube), then centrifuge at 10,000 rpm for 30 sec, discard flow-through.
6. Please do not take water-fast matter from the mixed solution into spin-column, otherwise it will block spin-column!
7. Add 500 µl buffer D centrifuge at 12,000 rpm for 30 sec, discard flow-through.
8. Add 500 µl buffer WB (please check ethanol added!), centrifuge at 12,000 rpm for 30 sec, and discard flow-through.
9. Place Spin-column AC back to collection tube, centrifuge at 13,000 rpm for 2 min and discard flow-through as much as possible, as ethanol inhibits the down-stream procedures.
10. Transfer the Spin-column AC to a clear 1.5 ml microcentrifuge tube, add 15 – 50 µl Buffer EB, let it stay at RT for 2-5 min, then centrifuge at 12,000 rpm for 2 min.

Buffer EB with volume less than 15 µl can affect elution efficiency and final DNA yield. Please add the elution of first time back to Spin-column AC and centrifuge again to obtain more DNA. pH of Buffer EB can affect elution efficiency. Please make sure pH is around 7.0-8.5 if elute DNA with water (adjust pH with NaOH). pH less than 7.0 will decrease elution efficiency.

Keep DNA at 2-8 °C; for long time storage, please keep at -20 °C.

Protocol 7: Isolate DNA from micro incision tissues (such as Formalin and Paraffin fixed tissues)

Please remove Paraffin before starting.

1. Add 10 µl Buffer A to a 0.2 microcentrifuge and then add micro incision tissue.
2. Add 10 µl Proteinase K (20mg/ml), vortex for 10 sec. Incubate at 56°C for 3 h or till thorough digestion and mix gently to help digest. Incubate at 56°C for 16 h for Formalin fixed samples.
3. Add 25 µl Buffer A, overturn to mix thoroughly, then add 50 µl Buffer C and 1 µl Carrier, vortex for 10 sec.
4. Add 50 µl 100% ethanol, overturn to mix thoroughly, incubate at RT for 5 min.
5. Transfer the mixture into a Spin-column AC (place the Spin -column AC to Collection Tube), then centrifuge at 10,000 rpm for 30 sec, discard flow-through.
6. Add 500 µl buffer D, centrifuge at 12,000 rpm for 30 sec, discard flow-through.
7. Add 500 µl buffer WB (please check ethanol added!), centrifuge at 12,000 rpm for 30 sec, and discard flow-through.
8. Place Spin-column AC back to collection tube, centrifuge at 13,000 rpm for 2 min and discard flow-through as much as possible, as ethanol inhibits the down-stream procedures.
9. Transfer the Spin-column AC to a clear 1.5 ml microcentrifuge tube, add 15 - 50 µl Buffer EB, let it stay at RT for 2-5 min, then centrifuge at 12,000 rpm for 2 min.

Buffer EB with volume less than 15 µl can affect elution efficiency and final DNA yield.

Please add the elution of first time back to Spin-column AC, and centrifuge again to obtain more DNA. pH of Buffer EB can affect elution efficiency. Please make sure pH is around 7.0-8.5 if elute DNA with water (adjust pH with NaOH). pH less than 7.0 will decrease elution efficiency.

Keep DNA at 2-8 °C; for long time storage, please keep at -20 °C.