BIO BASIC INC.

EZ-10 96 WELL PLATE GENOMIC DNA ISOLATION KIT (FOR ANIMAL)

BS4372 and BS437

Version 4.3
ISO9001 Certified

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Table of Contents

EZ-10 96 WELL PLATE GENOMIC DNA ISOLATION KIT (FOR ANIMAL)

Components	BS4372 2 Plates	BS437 5 Plates
ACL Solution	80 ml	200 ml
PBS Solution	80 ml	200 ml
AB Solution	80 ml	200 ml
Proteinase K	80 mg	200 mg
Wash Solution	2x24 ml	4x48 ml
Elution Buffer	20 ml	50 ml
EZ-10 96 Well Binding Plate	2	5
Deep Well Collection Plate	4	10
96 Well Storage Plate	2	5
Sealing Film	10	25
Protocol	1	1

1. ACL Solution may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution at 37ºC.
2. Before use, add 5ml of sterilized water to the tube containing 80mg of Proteinase K; or add 12.5ml of sterilized water to the tube containing 200mg of Proterinase K, keep at -20 ^{o}C for long term storage.
3. Before use, add 96ml of 100% ethanol to 24ml of Wash Solution, or add 192ml of 100% ethanol to 48ml of Wash Solution. If the volume of Wash Solution has changed due to leaking during transportation, it is necessary to re-

measure its volume, and adjust the volume of required ethanol accordingly (volume of added ethanol: volume of Wash Solution=4:1).

4. Elution Buffer is 2.0 mM Tris-HCl pH 8.0~8.5. Although TE buffer pH 8.0 or water can be used, yield is generally 10% lower.

Storage

With the exception of the Proteinase K, the kit may be stored at room temperature. The Proteinase K should be stored at -20ºC. The kit is stable for 12 months at room temperature. For longer storage, keep all contents cold.

Note

1. For centrifugation based method, there is a minimum height requirement of 75mm for apparatus to hold the assembly of EZ-10 96 Well Binding Plate and Deep Well Collection Plate.
2. Measure DNA quantity by UV absorption at A260 (1.0 OD unit is equivalent of 50 µg). Assess genomic DNA quality by an analytical 0.7% agarose gel. Isolated genomic DNA should not contain RNA. Its length should be over 50 kb.
3. Vacuum Manifold (SD5011) is sold separately.

Principle

This kit is designed for fast isolation of genomic DNA from animal tissues. The kit contains a membrane embedded EZ-10 96 Well Binding Plate for binding genomic DNA in each well. Nucleotides, proteins, salts, and other impurities are washed

away. Purified genomic DNA can be applied in most molecular biology experiments including restriction enzyme digestion, PCR, Southern-blotting and so on.

Applications
Genomic DNA purification from different animal tissues.

Features:
√ High yield
√ Rapid and economical
√ Preparation of high quality genomic DNA from various sources
√ No phenol / chloroform extraction , no ethanol precipitation required

Procedure for Isolation of Genomic DNA from Various Sources

Part A: For Animal Tissue
1. Cut up to 30 mg tissue and place in Deep Well Collection Plate.
2. Add 300 µl of ACL Solution (Animal Cell Lysis Solution) to Deep Well Collection Plate and 20µl of Proteinase K, seal it well with a Sealing Film.
3. Incubate at 55^{o}C until the tissue is completely lysed (usually 1-3 hours). Vortex occasionally. Incubate in shaking water bath can shorten lysis time.

4. Cool to room temperature. Vortex for 20 seconds and centrifuge at 5,700 x g for 5 minutes.

Centrifugation Based Procedure

5. Pipette 300µl of supernatant into an EZ-10 96 Well Binding Plate (if pellet not visible, repeat previous step) and add 300µl of AB Solution, seal, mix by inverting the EZ-10 96 Well Binding Plate several times, and keep for 2 minutes.
6. Place the 96 Well Binding Plate on top of a new Deep Well Collection Plate. Centrifuge at 5,700 x g for 5 minutes with a rotor for microtiter plates.
7. Discard the flow-through. Add 500 µl of Wash Solution to each well of the 96 Well Binding Plate and spin at 5,700 x g for 5 minutes. Discard flow-through and place EZ-10 96 Well Binding Plate back to the same Deep Well Collection Plate.
8. Repeat wash procedure in Step 7 (Optional: repeat wash procedure one more time if needed)
9. Discard flow-through and spin again for 5 minutes to remove residual Wash Solution.
10. Transfer the EZ-10 96 Well Binding Plate to a 96 Well Storage Plate. Add 30-50 µl Elution Buffer onto the centre of each well of the EZ-10 96 Well Binding Plate; incubate at 50 ^{o}C for 2 minutes. Centrifuge at 5,700 x g for 5 minutes.
11. Tightly seal the 96 Well Storage Plate. Genomic DNA is ready for use or store at -20^{o}C.

Vacuum Based Procedure

(For details, please see Vacuum Manifold Product Information SD5011)

5. Pipette 300µl of supernatant into an EZ-10 96 Well Binding Plate (if pellet not visible, repeat previous step) and add 300µl of AB Solution, seal, mix by inverting the EZ-10 96 Well Binding Plate several times, and keep for 2 minutes.
6. Assemble the Vacuum Manifold: Place Waste Tray in the Base, cover it with the Base Cap. Place the EZ-10 96 Well Binding Plate on top. Apply vacuum until the solution has passed through.
7. Discard the flow-through. Add 500 µl of Wash Solution to each well of the EZ-10 96 Well Binding Plate. Assemble the Vacuum Manifold as described in Step 6, apply vacuum until buffer has passed through.
8. Repeat wash procedure in step 7 (Optional: repeat wash procedure one more time if needed). After Wash Solution has been drawn through the column, apply maximum vacuum for additional 2 minutes to dry the membrane. If necessary, tap dry the bottom nozzle of EZ-10 96 well plate on paper towel before elution step.
9. For elution, assemble the Vacuum Manifold. This time, place a 96 Well Storage Plate Holder in the Base, and put the 96 Well Storage Plate on top, and cover it with Base Cap. Place EZ-10 96 Well Binding Plate on top securely. Mark the orientation appropriately.
10. To elute DNA, add 30-50 µl Elution Buffer onto the centre of each well of the EZ-10 96 Well Binding Plate; incubate at 50 ^{o}C for 2 minutes.

Apply vacuum for 1 minute. Switch off vacuum and ventilate vacuum manifold slowly.

11. Tightly seal the 96 Well Storage Plate. Genomic DNA is ready for use or store at -20°C.

Part B: For Rodent Tail

1. Place Deep Well Collection Plate in dry ice.
2. Cut 0.5cm to 1cm from end of tails and place in Deep Well Collection Plate.
3. Add 300 µl of ACL Solution (Animal Cell Lysis Solution) to Deep Well Collection Plate and 20 µl Proteinase K, seal with a Sealing Film.
4. Incubate at 55 °C overnight with rocking or for several hours with occasional mild vortexing every 15 minutes.
5. Cool to room temperature. Vortex for 20 seconds and centrifuge at 5,700 x g for 5 minutes.

Centrifugation Based Procedure

6. Pipette 300µl of supernatant into EZ-10 96 Well Binding Plate (if pellet not visible, repeat previous step) and add 300µl of AB Solution, seal, mix by inverting the EZ-10 96 Well Binding Plate occasionally, and keep for 2 minutes.
7. Place the 96 Well Binding Plate on top of a Deep Well Collection Plate. Centrifuge at 5,700 x g for 5 minutes with a rotor for microtiter plates.
8. Discard the flow-through. Add 500 µl Wash Solution to each well of the EZ-10 96 Well Binding Plate, spin at 5,700 x g for 5 minutes. Discard flow-through and place EZ-10 96 Well Binding Plate back to the same Deep Well Collection Plate.

9. Repeat wash procedure step 8 (Optional: repeat wash procedure one more time if needed).
10. Discard the flow-through and spin again for 5 minutes to remove residual Wash Solution.
11. Place the EZ-10 96 Well Binding Plate on top of a 96 Well Storage Plate. Add 30-50 µl Elution Buffer onto the centre of each well of the EZ-10 96-well plate; incubate at 50 ^{o}C for 2 minutes. Centrifuge at 5,700 x g for 5 minutes.
12. Tightly seal the 96 Well Storage Plate. Genomic DNA is ready for use or kept at – 20 ^{o}C.

Vacuum Based Procedure

(For details, please see Vacuum Manifold Product Information)

6. Pipette 300µl of supernatant into an EZ-10 96 Well Binding Plate (if pellet not visible, repeat previous step) and add 300µl of AB Solution, seal, mix by inverting the EZ-10 96 Well Binding Plate several times, and keep for 2 minutes.
7. Assemble the Vacuum Manifold, place a Waste Tray in the Base, cover it with the Base Cap, and put the EZ-10 96 Well Binding Plate on top. Apply vacuum until the solution has passed through.
8. Discard the flow-through. Add 500 µl of Wash Solution to each well of the 96 Well Binding Plate. Assemble the Vacuum Manifold as described in Step 7, apply vacuum until buffer has passed through.
9. Repeat wash procedure in step 8 (Optional: repeat wash procedure one more time if needed). After Wash Solution has been drawn

through the column, apply maximum vacuum for additional 2 minutes to dry the membrane. If necessary, tap dry the bottom nozzle of EZ-10 96 well plate on paper towel before elution step.

10. For elution, assemble the Vacuum Manifold. This time, place a 96 Well Storage Plate Holder in the Base, and put the 96 Well Storage Plate on top, and cover it with Base Cap. Place EZ-10 96 Well Binding Plate on top securely. Mark the orientation appropriately.
11. To elute DNA, add 30-50 µl Elution Buffer onto the centre of each well of the EZ-10 96 Well Binding Plate; incubate at 50 ^{o}C for 2 minutes. Apply vacuum for 1 minute. Switch off vacuum and ventilate vacuum manifold slowly.
12. Tightly seal the 96 Well Storage Plate. Genomic DNA is ready for use or store at -20^{o}C.

Part C: For Cultured Animal Cell

1. Centrifuge the appropriate number of cells(>5x10^{6}) for 5 minutes at 5,700 x g.
2. Resuspend pellet in 500 µl of PBS Solution.
3. Wash the cells 2 times with PBS.
4. Resuspend pellet in 300µl of ACL Solution Buffer.
5. Add 20µl of Proteinase K.
6. Incubate at 55^{o}C for 10 minutes.
7. Cool to room temperature. Vortex for 20 seconds and centrifuge 5,700 x g for 5 minutes.

Centrifugation based procedure

8. Pipette 200 µl of supernatant into an EZ-10 96 Well Binding Plate (if pellet not visible, repeat previous step) and add 200 µl AB Solution. Mix

by inverting the EZ-10 96 Well Binding Plate occasionally, and keep for 2 minutes.

9. Place the 96 Well Binding Plate on top of a fresh Deep Well Collection Plate. Centrifuge at 5,700 x g for 5 minutes and discard the flow-through.
10. Add 500 µl of Wash Solution to each well of the EZ-10 96 Well Binding Plate, and spin at 5,700 x g rpm for 5 minutes. Discard flow-through and place EZ-10 96 Well Binding Plate back to the same Deep Well Collection Plate.
11. Repeat washing procedure step 10 (Optional: repeat wash procedure one more time if needed).
12. Discard flow-through and spin again for 5 minutes to remove residual Wash Solution.
13. Transfer the EZ-10 96 Well Binding Plate to a 96 Well Storage Plate. Add 30-50 µl Elution Buffer onto the centre of each well of the EZ-10 96 Well Binding Plate; incubate at 50 ^{o}C for 2 minutes. Centrifuge at 5,700 x g for 5 minutes.
14. Genomic DNA is ready for use or kept at -20 ^{o}C.

Vacuum Based Procedure

(For details, please see Vacuum Manifold Product Information)

8. Pipette 200µl of supernatant into an EZ-10 96 Well Binding Plate (if pellet not visible, repeat previous step) and add 200µl of AB Solution, seal, mix by inverting the EZ-10 96 Well Binding Plate several times, and keep for 2 minutes.
9. Assemble the Vacuum Manifold, place a Waste Tray in the Base, cover it with the Base Cap, and put the EZ-10 96 Well Binding Plate on top.

Apply vacuum until the solution has passed through.

10. Discard the flow-through. Add 500 µl of Wash Solution to each well of the 96 Well Binding Plate. Assemble the Vacuum Manifold as described in Step 9, apply vacuum until buffer has passed through.
11. Repeat wash procedure in step 10 (Optional: repeat wash procedure one more time if needed). After Wash Solution has been drawn through the column, apply maximum vacuum for additional 2 minutes to dry the membrane. If necessary, tap dry the bottom nozzle of EZ-10 96 well plate on paper towel before elution step.
12. For elution, assemble the Vacuum Manifold. This time, place a 96 Well Storage Plate Holder in the Base, and put the 96 Well Storage Plate on top, and cover it with Base Cap. Place EZ-10 96 Well Binding Plate on top securely. Mark the orientation appropriately.
13. To elute DNA, add 30-50 µl Elution Buffer onto the centre of each well of the EZ-10 96 Well Binding Plate; incubate at 50 ^{o}C for 2 minutes. Apply vacuum for 1 minute. Switch off vacuum and ventilate vacuum manifold slowly.
14. Tightly seal the 96 Well Storage Plate. Genomic DNA is ready for use or store at -20^{o}C.

Part D: From Paraffin Tissue

1. Excise 25~30mg paraffin tissue with a clean, sharp scalpel. And transfer to a Deep Well Collection Plate.

2. Add 1.2ml xylene (self-prepared by user) to Deep Well Collection Plate, seal, and then vortex for 3 minutes. Xylene is used to remove paraffin.
3. Centrifuge at 5,700 x g for 5 minutes at room temperature.
4. Remove the supernatant completely. Keep the pellet.
5. Add 1.2ml 100% of ethanol to Deep Well Collection Plate, seal, gently vortex for 1 minute. Incubate at room temperature for 1 minute.
6. Centrifuge at 5,700 x g for 5 minutes at room temperature. Discard supernatant completely.
7. Repeat washing step once from step 4 to 6.
8. Incubate at 37 ^{o}C for 10-15 minutes to remove residual ethanol.
9. Resuspend the sample in 200µl TE buffer, and continue immediately with Step 10**.**
10. Add 300 µl of ACL Solution (Animal Cell Lysis Solution) to Deep Well Collection Plate and add 20µl Proteinase K, then seal it.
11. Incubate at 55^{o}C until the tissue is completely lysed (usually 1-3 hours). Vortex occasionally.
12. Cool to room temperature. Vortex for 20 seconds and centrifuge at 5,700 x g for 5 minutes.

Centrifugation based method

13. Pipette 300 µl of supernatant into an EZ-10 96 Well Binding Plate (if pellet not visible, repeat previous step) and add 300 µl of AB Solution, Seal. Mix by inverting the EZ-10 96 Well

Binding Plate occasionally, and keep for 2 minutes.

14. Place the 96 Well Binding Plate on top of a fresh Deep Well Collection Plate. Centrifuge at 5,700 x g for 2 minutes and discard the flow-through.
15. Add 500 µl of Wash Solution, and spin at 5,700 x g for 5 minutes. Discard flow-through and place EZ-10 96 Well Binding Plate back to the same Deep Well Collection Plate.
16. Repeat washing step 15 (Optional: repeat wash procedure one more time if needed).
17. Discard flow-through. Spin at 5,700 x g for 5 minutes to remove any residual Wash Solution.
18. Place the EZ-10 96 Well Binding Plate on top of a new 96 Well Storage Plate. Add 30-50 µl Elution Buffer onto the centre of each well of the EZ-10 96 Well Binding Plate. Incubate the plate at 37^{o}C or 50 ^{o}C for 2 minutes could increase the recovery yield.
19. Spin at 5,700 x g for 5 minutes to elute DNA from the column.
20. Tightly seal the 96 Well Storage Plate. Genomic DNA is ready for use or store at -20^{o}C

Vacuum Based Procedure

(For details, please see Vacuum Manifold Product Information)

13. Pipette 300µl of supernatant into an EZ-10 96 Well Binding Plate (if pellet not visible, repeat previous step) and add 300µl of AB Solution, seal, mix by inverting the EZ-10 96 Well Binding Plate several times, and keep for 2 minutes.

14. Assemble the Vacuum Manifold, place a Waste Tray in the Base, cover it with the Base Cap, and put the EZ-10 96 Well Binding Plate on top. Apply vacuum until the solution has passed through.
15. Discard the flow-through. Add 500 µl of Wash Solution to each well of the 96 Well Binding Plate. Assemble the Vacuum Manifold as described in Step 14, apply vacuum until buffer has passed through. Discard the flow-through.
16. Repeat wash procedure in step 15. After Wash Solution has been drawn through the column, apply maximum vacuum for additional 2 minutes to dry the membrane. If necessary, tap dry the bottom nozzle of EZ-10 96 well plate on paper towel before elution step.
17. For elution, assemble the Vacuum Manifold. This time, place a 96 Well Storage Plate Holder in the Base, and put the 96 Well Storage Plate on top, and cover it with Base Cap. Place EZ-10 96 Well Binding Plate on top securely. Mark the orientation appropriately.
18. To elute DNA, add 30-50 µl Elution Buffer onto the centre of each well of the EZ-10 96 Well Binding Plate; incubate at 50 ^{o}C for 2 minutes. Apply vacuum for 1 minute. Switch off vacuum and ventilate vacuum manifold slowly.
19. Tightly seal the 96 Well Storage Plate. Genomic DNA is ready for use or store at -20^{o}C.

PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC RESEARCH ONLY!
NOT INTENDED FOR HUMAN OR ANIMAL USE!

Other Kits Available

EZ-10 Spin Column Plasmid DNA MiniPreps Kit (BS413, BS414)

EZ-10 Spin Column PCR Products Purification Kit (BS363, BS364)

EZ-10 Spin Column DNA Gel Extraction Kit (BS353, BS354)

EZ-10 96-Well Spin Column PCR Products Purification Kit (BS365, BS3652)

EZ-10 96 Well Spin Column Plasmid DNA Minipreps Kit (BS415, BS4152)

And much more…….

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