PRODUCT INFORMATION

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EZ96-205 96-well Genomic DNA miniprep kit (for animal tissues and cell culture):

Components	EZ96-205 , 2 Plates	EZ96-205 , 5 Plates
ACL Solution[A]	80 ml	200 ml
PBS Solution	80 ml	200 ml
AB solution	80 ml	200 ml
WASH Solution[C]	2x35	4x48 ml
Elution Buffer[D]	20 ml	50 ml
Proteinase K (lyophilized powder)[B]	80 mg	200 mg
EZ96DBP plates (DNA Binding plates)	2	5
EZ96DWP plate (2ml deep well collection plates)	4	10
96 Storage plate	2	5
Sealing film	10	25
Protocol	1	1

A. ACL Solution may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution at 37ºC.
B. Before use, add 5 ml of sterilized water to the tube containing 80 mg of proteinase K, keep at -20°C for long term storage.
C. Before use, add 140 ml of 100% ethanol to 35 ml Wash Solution.
D. Elution Buffer is 2.0 mM Tris-HCl, pH 8.0~8.5. TE buffer (pH 8.0) or water may be used instead.

Storage: With the exception of proteinase K, the kit may be stored at room temperature. Proteinase K should be stored at 4ºC for short term. The kit is stable for 18 months at room temperature. For longer storage, keep all contents cold.
Note: The purification method is based on centrifugation. A vacuums manifold can be used as well.

Principle

This kit is designed for fast isolation of genomic DNA from animal tissues and cultured cells. The kit contains DNA Binding Plates (EZ96DBP), in which a silica membrane is embedded in each well for binding genomic DNA. Nucleotides, proteins, salts, and other impurities do not bind to the membrane. Purified genomic DNA can be used in most molecular biology experiments including restriction enzyme digestions, PCR, Southern blotting etc.

Features:

*****Fast, high-throughput, low cost, high yield, and high quality genomic DNA isolation from variable sources.
* No phenol/chloroform extraction, no ethanol precipitation.

Procedure:

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 to Deep Well Collection Plate and 20 µl proteinase K, then seal with sealing film.
3. Incubate at 55°C until the tissue is completely lysed (usually 1-3 hours). Occasionally vortex. Incubation in shaking water bath can reduce lysis time.
4. Cool to room temperature. Vortex for 20 seconds and Centrifuge at 5,700 xg for 5 minutes.
5. Pipette **300** µl of supernatant into a DNA Binding plate (EZ96DBP) and add **300** µl of AB Solution, seal, mix by inverting the Plate several times, and keep for 2 minutes.
6. Place the binding plate on top of a new Deep Well Collection Plate. Centrifuge at 5,700 xg for 5 minutes with a rotor for microtiter plates.
7. Discard flow-through. Add 500 µl Wash Solution to each well of binding plate and spin at 5,700 xg for 5 minutes. Discard flow-through and place the binding plate back to the same Deep Well Collection Plate. Repeat this wash step once more time.
8. Discard flow-through and spin again for 5 min to remove residual wash solution.
9. Transfer the binding plate to a 96-well storage plate. Add 30-50 µl Elution Buffer onto the center of each well of binding plate; incubate at 50°C for 2 minutes. Centrifuge at 5,700 xg for 5 minutes.
10. Tightly seal the 96-well storage plate and store at -20°C. Genomic DNA is ready to use.

B. For Rodent Tail

1. Place Deep Well Collection Plate in dry ice.

2. Cut 0.5 cm to 1 cm from end of tails and place in Deep Well Collection Plate.
3. Add 300 µl of ACL Solution to Deep Well Collection Plate and 20 µl proteinase K, then seal with 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 20 seconds and Centrifuge at 5,700 xg for 5 minutes.
6. Follow Steps 5-10 in **A** (**For Animal Tissue).**

C. For Cultured Animal Cells

1. Centrifuge the appropriate number of cells (>$5x10^6$) for 5 minutes at 5,700 xg.
2. Resuspend the pellet in 500 µl of PBS solution.
3. Wash the cells 2 times with PBS.
4. Resuspend pellet in 300 µl of ACL solution buffer. Add 20 µl of proteinase K.
5. Incubate at 55°C for 10 minutes.
6. Cool to room temperature. Vortex for 20 seconds and Centrifuge at 5,700 xg for 5 minutes.
7. Pipette **200** µl of supernatant into a DNA Binding plate (EZ96DBP) and add **200** µl of AB Solution, seal, mix by inverting the Plate several times, and keep for 2 minutes.
8. Follow Steps 6-10 in **A** (**For Animal Tissue).**

D. From Paraffin Tissue

1. Excise 25~30 mg paraffin tissue with a clean, sharp scalpel. And transfer to a Deep Well Collection Plate.
2. Add 1.2 ml 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 xg for 5 minutes at room temperature. Remove the supernatant completely. Keep the pellet.
4. Add 1.2 ml 100% of ethanol to Deep Well Collection Plate, Seal, Gently vortex for 1min. Incubate at room temperature for 1 minute. Centrifuge at 5,700 xg for 5 minutes at room temperature. Discard supernatant completely. Repeat this washing step once.
5. Incubate at 37°C for 10-15 minutes to remove residual ethanol.
6. Resuspend the sample in 200 µl TE buffer, and continue immediately with next Step (Step 7)**.**
7. Add 300 µl of ACL Solution to Deep Well Collection Plate and add 20 µl proteinase K, seal it with sealing film.
8. Incubate at 55°C until the tissue is completely lysed (usually 1-3 hours). Occasionally vortex.
9. Cool to room temperature. Vortex for 20 seconds and Centrifuge at 5,700 xg for 5 minutes.
10. Follow Steps 5-10 in **A** (**For Animal Tissue).**

E. From Blood Samples

1. For fresh or frozen anticoagulated mammal blood sample (nonnucleated erythrocytes), add 270 µl blood sample to each well of a deep well collection plate (EZ96DWP), add 15 µl of 10% SDS (final 0.5%), seal with sealing film and mix thoroughly by converting the plate for several times. **NOTE**: If using <270 µl blood sample, adjust the sample volume to 270 µl using PBS and then add SDS. Continue with step 3.
2. For fresh or frozen nucleated erythrocytes (blood from birds, fish or frogs), add 5-10 µl anticoagulated blood to ~300 µl ALC buffer+proteinase K in a deep well collection plate (EZ96DWP), seal with sealing film and mix thoroughly by votexing or pipetting to yield homogeneous solution.
   NOTE: Add 20 µl RNaseA (20 mg/ml) if RNA-free genomic DNA is required. Mix well and incubate at room temperature for 2 min before continuing with step 3.
3. Incubate at 55°C for 10-20 min to promote protein digestion.
4. When lysis completes, cool to room temperature, votex for 20 seconds and centrifuge at 5,700 xg for 5 min.
5. Pipette supernatant into a DNA binding plate (EZ96DBP) and add equal volume of AB solution, seal, mix by inverting the Plate several times, and keep for 2 minutes.
6. Place the binding plate on the top of a new Deep Well Collection Plate. Centrifuge at 5,700 xg for 5 minutes with a rotor for microtiter plates.
7. Discard flow-through. Add 500 µl Wash Solution to each well of binding plate and spin at 5,700 xg for 5 minutes. Discard flow-through and place the binding plate back to the same Deep Well Collection Plate. Repeat this wash step once more time.
8. Discard flow-through and spin again for 5 min to remove residual wash solution.
9. Transfer the binding plate to a 96-well storage plate. Add 30-50 µl Elution Buffer onto the center of each well of binding plate; incubate at 50°C for 2 minutes. Centrifuge at 5,700 xg for 5 minutes.
10. Tightly seal the 96 well storage plate and store at -20°C. Genomic DNA is ready to use.