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PRODUCT INFORMATIOIN

Mini Column Genomic DNA Isolation kit for Animal Tissues and Mouse Tail
(Cat#EZC205)

Kit Contents and Storage Conditions:

Components	EZC205 (50 preps)	*Storage*
ACL Solution	20ml	RT (room temperature)
PBS Solution	20ml	RT
AB solution*	20ml	RT
WASH Solution	12ml	RT
Elution Buffer	5ml	RT
ProteinaseK(20mg, lyophilized powder)	20mg	-20^{o}C
DNA Mini Spin Column/ with collection tubes*	50	RT

***Mini Spin columns can be ordered separately for leftover solutions (cat#EZC101, $39 for 100 columns)**

Safety Information:
AB Solution contains guanidine hydrochloride: harmful, irritant. Risk and safety phrases:* R22-36/38, S13-26-36-46
proteinase K: sensitizer, irritant. Risk and safety phrases:* R36/37/38-42/43, S23-24-26-36/37

Description:
This kit is designed for fast isolation of genomic DNA from animal tissue (including mouse tails) without organic solvent extraction or ethanol precipitation. The typical size is ranged from 100bp-50kb. The kit contains a membrane embedded DNA mini spin column for binding up to 50μg of DNA and for removing salts, enzymes, proteins, and all other impurities. The recommended sample size <5x10^{6} cell and <20mg tissue (for genotyping with mouse tail: <0.3 cm, rat tail: <0.2cm). For recovery genomic DNA from very samples (e.g. 0.1cm mouse tail or <100 cells), please use Tini spin columns (Cat#EZ106) with same solutions but reduce the volume proportionally by 1 /2 or 1/3, and elute the genomic DNA in 5-20μl volume. The kit for small amount genomic DNA prep is also available (Cat# 601, for 50 preps).

Applications:
1. Genomic DNA preparation from different sources:
   (a) Blood (b) Various animal tissues (c) Mouse and Rat tails.
2. Highly purified genomic DNA is ready to use in the following applications:
(a)PCR (b) Southern blot (c) Analysis by pulsed-field electrophoresis (d) Restriction enzymes digestion

Features:
√ Fast and High yield √ No phenol / chloroform extractions √ No ethanol precipitation
√ Yields fully hydrated genomic DNA √ Columns are sold separately for leftover solutions

Procedures for isolation of Genomic DNA from Animal Tissues/Tails
This protocol is designed for isolation of genomic DNA from animal tissues and mouse tails using DNA Mini Spin Column. If you have small samples (see Table above), you can use Tini spin Column (cat# EZC106) with same protocol but cut down all the solutions to1/2 to 1/3 or buy kit (Cat#601). Usually mouse tails have insignificant amount of RNA, so RNase A digestion can be omitted. Transcriptional active tissues such as liver and kidney contain high levels of RNA, RNase A may be used to digest RNA (before adding BD buffer) to remove RNA. RNA will not affect PCR as downstream application!

Important Notes:
In order to obtain optimal genomic DNA yield and purity, it is essential to use the correct amount of starting material. Here is the recommendation for maximum amounts of starting material:

Sample	Amount for Mini Spin Column	Amount for Mini Spin Column
Animal tissue	25mg	10mg
Mammalian Blood	100 μl	
Bird or fish blood (with nucleated erythrocytes)	10 μl	
Mouse Tails (for genotyping)	0.3cm	0.1cm
Rat Tails (for genotyping)	0.3cm	0.1cm
Cultured Cells	5x106	5x105
Bactuerial	2x109	

Animal tissue size and estimated weight: for most animal tissues

Tissue size (LxW) (mm)	Thickness (mm)	Estimated weight (mg)
1.5mmx1.5mm	1-2mm	1mg
1.7mmx1.7mm	1-2mm	3mg
2.0mmx2.0mm	1-2mm	5mg
2.5mmx2.5mm	1-2mm	7mg
3.0mmx3.0mm	1-2mm	10mg
3.5mmx3.5mm	1-2mm	15mg

Things to do before starting:

1. Buffer ALC may form precipitates upon storage. Warm to 55^{o}C if necessary.
2. Add 1ml sterilized water to proteinase K to make stock solution at 20mg/ml (keep at -80^{o}C for long term storage).
3. Add 48ml of 100% ethanol to 12ml of WASH buffer.
4. If using frozen tissue, equilibrate the sample to room temperature. Avoid freeze/thaw cycle of samples since this will lead to reduced DNA size.
5. Prepare ALC+proteinase K mixture: add 100μl of proteinase K stock into1.5ml ALC. This is good for 5 DNA preps for tails.

Procedures:

1. Cut up to 0.3cm mouse tails and place in a 1.5ml microcentrifuge tube, For tissue samples, cut up to 20mg tissue (up to 10mg spleen) into small pieces and place into tube.
2. Add 300μl ALC buffer+proteinase K and incubate the tube at 55^{0}C till tissue or tail is completely lysed (2h to overnight). Ensure the tissue is completely immersed in the buffer mix. (Option to treat the sample with RNase A (not included): 4ul of 100mg/ml to each tube and incubate for 2 min at room temperature).
3. When lysis completes, cool to room temperature, votex for 20 seconds and centrifuge at full speed for 2 min.
4. Pipette 250μl of supernatant into Mini spin Column and add equal volume (250μl) of AB solution. Mix by inverting the column 4-6 times, and then centrifuge in a microcentrifuge for 1 min at full speed. Discard flow-through.
5. Add 500ul of WASH buffer (ethanol added) to the column and centrifuge at full speed for 1 minute. Discard flow-through and repeat this step one more time.
6. Centrifuge the tube for 2 minutes to remove any residual wash solution (**This step is important! Trace amount of ethanol may interfere with downstream applications**).
7. Place the column into a clean 1.5ml centrifuge tube.
8. Add 50-100μl ddH_2O (preheated at 65^{0}C) to the center of the column and incubate at room temperature for 5 min. Centrifuge at 10,000xg (~13,000rpm) for 1 minute to elute the genomic DNA. Additional elution step may need for better recovery of genomic DNA.

Procedures for isolation of Genomic DNA from Animal/Human Cell and Blood Lysate.

This protocol is design for isolation of genomic DNA from animal/human cell and blood lysate using DNA Mini spin column (Cat#EZC101). If you have small samples (see table above), you can use Tini Spin Column (Cat#EZC106) with same protocol but cut down all the solutions to 1/2- 1/3, or buy kit (cat# Cat#601).

Cell numbers in Different Sizes of Multiwall Culture Plates and Dishes

Multiwell plates	Number of Cells	Culture Dishes	Number of Cells
96-well	4-5x10^{4}	35mm dish	1x10^{6}
48-well	1x10^{5}	60mm dish	2.5x10^{6}
24-well	2.5x10^{5}	100mm dish	7x10^{6} to 1x10^{7}
12-well	5x10^{5}	145-150mm	2x10^{7}
6-well	>1x10^{6}	40-50ml Flask	3x10^{6}
		250-300 ml Flask	1x10^{7}
		650-750ml Flask	2x10^{7}

1. For adherent cells (up to 5x10^{6}), remove the medium and harvest cells by trypisinization or method of your choice. Wash the cells with 500μl PBS solution. Resuspend cells in 300 μl ACL buffer+ proteinase K.
2. For suspension cells (up to 5x10^{6}), harvest the cell by centrifugation. Resuspend cells in 300 μl ACL buffer+proteinase K.
3. For fresh or frozen anticoagulated mammal blood sample (nonnucleated erythrocytes), add 270 μl blood sample to a sterile microcentrifuge tube, add 15μl of 10% SDS (final 0.5%), mix thoroughly by votexing or pipetting to yield homogeneous solution. If using <270 μl blood sample, adjust the sample volume to270 μl using PBS and then add SDS.
4. For fresh or frozen nucleated erythrocytes (blood from birds, fish or frogs), add 5-10ul anticoagulated blood to ~300 μl ALC buffer+proteinase K, and mix thoroughly by votexing or pipetting to yield homogeneous solution.
5. Option to treat the sample with RNase A: add 4ul of 100mg/ml to each tube and incubate for 2 min at room temperature.
6. Incubate at 55^{0}C for 10-20min to promote protein digestion.
7. Follow step 3-8 from the protocol above.