

Enzymax LLC

Tel: 859-219-8482 Fax: 859-887-9135 Web: www.enzymax.net

PRODUCT INFORMATION

Purification of Genomic DNA from Small Animal Tissue/Tail samples (Cat#601)

Kit Contents and Storage Conditions:

| Components | 601 (50 preps) | Storage |
|---|----------------|-----------------------|
| ACL Solution | 10ml | RT (room temperature) |
| PBS Solution | 20ml | RT |
| AB solution* | 10ml | RT |
| WASH Solution | 6ml | RT |
| Elution Buffer | 2.5ml | RT |
| ProteinaseK (20mg, lyophilized powder) | 10mg | -20°C |
| DNA Tini Spin Column/ with collection tubes | 50 | RT |

*Tini Spin columns can be ordered separately for leftover solutions (cat#EZC106N, \$42 for 50 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 small amount of 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 Tini spin column for binding up to 20µg of DNA and for removing salts, enzymes, proteins, and all other impurities. The recommended sample size 5×10^5 cell and 5mg tissue (for genotyping with mouse tail: <math><0.2</math> cm, rat tail: <math><0.1</math>cm). This kit can also be used for concentration of genomic DNA since the elution volume of Tini Spin Column can as low as 5µl. For larger sample use kit EZC205 with Mini Spin Columns or buy bulk DNA Mini Spin Column (EZC101, \$38 for 100 columns)

Applications:

1. Genomic DNA preparation from different sources:
(a) Blood (b) Various animal tissues (c) Mouse and Rat tails. (D) Saliva (E) water sample
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 small amount of Animal Tissues/Tails

This protocol is designed for isolation of genomic DNA from small amount of animal tissues and mouse tails using DNA Tini Spin Column. 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). RNA does not affect PCR.

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 | 5×10^6 | 5×10^5 |
| Bacterial | 2×10^9 | |

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°C if necessary.
2. Add 1ml sterilized water to proteinase K to make stock solution at 20mg/ml (keep at -80°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 into 1.5ml ALC. This is good for 5 DNA preps for tails.

Procedures:

1. Cut up to 0.1cm mouse tails and place in a 1.5ml microcentrifuge tube, For tissue samples, cut up to 10mg tissue (up to 5mg spleen) into small pieces and place into tube.
2. Add 140µl ALC buffer+proteinase K and incubate the tube at 55°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): 2ul of 100mg/ml to each tube and incubate for 2 min at room temperature).
3. When lysis completes, cool to room temperature, vortex for 20 seconds and centrifuge at full speed for 2 min.
4. Pipette 120µl of supernatant into Tini spin Column and add equal volume (120µl) 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 125ul 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 additional minute to remove any residual wash solution (**This step is important**).
7. Place the column into a clean 1.5ml centrifuge tube.
8. Add 10-50µl ddH₂O (preheated at 65°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 Small Amount of Animal/Human Cell and Blood.

This protocol is design for isolation of genomic DNA from small amount of animal/human cell and blood lysate using DNA Tini spin column (Cat#EZC106)

Cell numbers in Different Sizes of Multiwell 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 5x10⁵), remove the medium and harvest cells by trypsinization or method of your choice. Wash the cells with 250µl PBS solution. Resuspend cells in 150 µl ACL buffer+ proteinase K.
2. For suspension cells (up to 5x10⁵), harvest the cell by centrifugation. Resuspend cells in 150 µl ACL buffer+proteinase K.
3. For fresh or frozen anticoagulated mammal blood sample (nonnucleated erythrocytes), add 25-50 µl blood sample to a sterile microcentrifuge tube. If using <50 µl blood sample, adjust the sample volume to 50 µl using PBS. Add 150 µl ACL buffer+ proteinase K, and mix thoroughly by vortexing or pipetting.
4. For fresh or frozen nucleated erythrocytes (blood from birds, fish or frogs), add 5µl anticoagulated blood to ~150 µl ALC buffer+proteinase K, and mix thoroughly by vortexing or pipetting to yield homogeneous solution.
5. Option to treat the sample with RNase A: add 2µl of 100mg/ml to each tube and incubate for 2 min at room temperature.
6. Incubate at 55°C for 10-20min to promote protein digestion.
7. Follow step 3-8 from the protocol above.

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