UE Multisource Genomic DNA Miniprep Kit

This system employs a special lysis Buffer and Proteinase K, to efficiently release genomic DNA from the biologic starting materials. Each Miniprep column will bind and purify up to 20 μg of genomic DNA, is suitable for a variety of applications, such as PCR amplification, Southern blot analysis, etc.

1. Kit contents, storage and stability

Cat. No.	UE-MN-MS-GDNA-10	UE-MN-MS-GDNA-50	UE-MN-MS-GDNA-250
Kit size	**10 preps**	**50 preps**	**250 preps**
Miniprep column	**10**	**50**	**250**
2 ml microfuge tube	**20**	**100**	**500**
1.5 ml microfuge tube	**10**	**50**	**250**
RNase A	**12 μl**	**60 μl**	**300 μl**
Buffer C-L	**2 ml**	**9 ml**	**45 ml**
Proteinase K	**4 mg**	**18 mg**	**90 mg**
PK Buffer	**260 μl**	**1.2 ml**	**6 ml**
Buffer P-D	**5 ml**	**25 ml**	**125 ml**
Buffer W1	**6 ml**	**30 ml**	**145 ml**
Buffer W2 concentrate	**5 ml**	**24 ml**	**120 ml**
Eluent	**3 ml**	**12 ml**	**60 ml**
Protocol manual	**1**	**1**	**1**

RNase A: 50 mg/ml. Store at room temperature. Buffer C-L: Lysis buffer. Store at room temperature.

Proteinase K: Lyophilized Proteinase K is stable for up to 6 months after delivery when stored at room temperature. After reconstitution with PK Buffer, Proteinase K is stable for 2 months when stored at 4°C. Storing the Proteinase K stock solution at room temperature for prolonged periods of time should be avoided.

PK Buffer: Used to resuspend Proteinase K. Store at room temperature.

Buffer P-D: Protein precipitation buffer. Store at room temperature.

Buffer W1: Wash buffer. Store at room temperature.

Buffer W2 concentrate: Desalting buffer. Before using the kit, add the amount of ethanol specified on the bottle label to the Buffer W2 concentrate. Either 100% or 95% denatured ethanol can be used. Store at room temperature.

Eluent: 2.5 mM Tris-HCl, pH 8.5. Store at room temperature.

2. Caution

Buffer C-L, Buffer P-D and Buffer W1 contain chemical irritants. When working with these buffers, always wear suitable protection such as safety glasses, laboratory coat and gloves. Be careful to avoid contact with eyes and skin. In the case of such contact, wash immediately with water. If necessary, seek medical assistance.

3. Preparation before experiment

1) Before using the kit, add the amount of absolute ethanol specified on the Buffer W2 label and mix well.
2) Dissolve proteinase K in PK buffer according to the label on the bottle, do not vortex.
3) Adjust a water bath to 56°C.
4) Check Buffer C-L and for precipitation before each use. If precipitation occurs, incubate at 37°C to dissolve the precipitate.
5) Pre-warming the Eluent to 65°C will often improve elution efficiency.

4. Protocols

Please choose the corresponding steps 1-5 according to different samples, homogenization, lysis and removal of protein and other impurities.

A. Purification of Genomic DNA from Animal Tissues

1. Select 1-20 mg of animal tissue and transfer to a mortar, pre-chilled on ice. Grind rapidly and vigorously to form a homogenate.

   Note:** For the following tissue types that require freezing in liquid nitrogen, perform the following steps after crushing: Add 350 µl PBS and 0.9 µl RNase A. Heat the mortar to 56 °C in a water bath until the PBS has just melted. Grind gently for 1 minute. Then ***proceed to Step 3, below.

   - DNase-rich tissues, such as pancreas, thymus, lymphoid tissue, etc.
   - Collagen-rich tissues, such as skin, connective tissue, etc.
   - Keratoprotein-rich tissues or hard tissues, such as bone.
2. Add 350 µl of phosphate-buffered saline (PBS, not provided) and 0.9 µl of RNase A. Gently grind for 30 seconds to homogenously mix the PBS with the ground tissue.
3. Collect 350 µl of the homogenate and transfer to a 2 ml microfuge tube. If the volume of the homogenate is less than 350 µl, make it up to 350 µl with PBS.
4. Add 20 µl Proteinase K and 150 µl Buffer C-L. Mix immediately by vortexing for 1 minute. Briefly centrifuge to remove drops from inside the lid and incubate at 56°C for 10 minutes.

   Note: Do not add Proteinase K directly to buffer C-L.
5. Add 350 µl Buffer P-D to the sample and mix by vortexing at top speed for 30 seconds. Centrifuge for 10 minutes at 12,000xg at ambient temperature to pellet cellular debris.

B. Purification of Genomic DNA from Plant Tissues

1. Using Table 1 (below), weigh out the appropriate amount of either fresh plant tissue and transfer to a mortar. The amount should be reduced by half if lyophilized, dehydrated, or dried tissues are used. Carefully add liquid nitrogen directly to the sample until it is completely frozen. Use the pestle to pulverize it quickly and vigorously until it is reduced to a fine powder. Add liquid nitrogen as required to prevent the material from thawing during pulverization. After pulverizing, warm the mortar to 56°C in a water bath until the pulverized material just melts.

*__Table 1.__ Types of fresh plant tissues used for genomic DNA preparation

Flower or leaves	10-100 mg
Plant stem	≤ 240 mg
Plant root	≤ 240 mg
Plant seed	≤ 240 mg

* **Note:** Incomplete grinding will reduce the yield of genomic DNA.

* **Note**: If cultured plant cells are used, collect $2x10^3$-$1x10^7$ plant cells and spin for 1 minute at 10,000xg to pellet the cells. Resuspend the plant cells in 150 µl of PBS and transfer to the mortar. Carefully add liquid nitrogen directly to the sample until it is completely frozen. Use the pestle to pulverize it quickly and vigorously until it is reduced to a fine powder. Add liquid nitrogen as required to prevent the material from thawing during pulverization. After pulverizing, warm the mortar to 56°C in a water bath until the pulverized material just melts. Proceed to Step 2, below.

2. Add 350 µl of PBS (not provided), and 0.9 µl of RNase A. Quickly and vigorously for 30 seconds to homogenously mix the PBS with the ground tissue.

   * **Note**: When the weight of the fresh plant tissue is >120 mg or the dried plant tissue is >60 mg, add 700 ul of PBS. After Step 2 has been completed, divide the sample evenly between two 2 ml microfuge tubes. Steps 4-5 will proceed in two parallel 2 ml microfuge tubes. In Step 6, the contents of the two tubes will be consolidated into a single Miniprep column.

3. Transfer the tissue homogenate into a 2 ml microfuge tube (provided). Determine the approximate volume. If the volume of the homogenate is less than 350 µl, make it up to 350 µl with PBS.

4. Add 20 µl Proteinase K and 150 µl Buffer C-L. Mix immediately by vortexing for 1 minute. Briefly centrifuge to remove drops from inside the lid and incubate at 56°C for 10 minutes.

   * **Note**: Do not add Proteinase K directly to buffer C-L.

   * **Note**: If fibrous samples such as plant stem and root, or starch- and protein-rich samples such as seeds are used, increase the incubation time to 30 minutes in the water bath.

5. Add 350 µl Buffer P-D to the sample and mix by vortexing at top speed for 30 seconds. Centrifuge for 10 minutes at 12,000xg at ambient temperature to pellet cellular debris.

C. Purification of Genomic DNA from Cultured Animal Cells, Lymphocytes, Bone Marrow, Dried Blood Spot and Bone.

Lysis and homogenization of sample

Select method A-F, depending upon the type of starting material used. If genomic DNA is extracted from plant cells, please follow the previous protocol "Purification of Genomic DNA from Plant Tissues" (above) to homogenize the plant cells.

a. Cells grown in suspension or a cell suspension freshly isolated from animal tissues:

1a. Collect $1x10^3$-$2x10^6$ cells in suspension and transfer to a 2 ml microfuge tube. Centrifuge for 5 minutes at 2,000xg to pellet the cells. Discard the supernatant.

2a. Add 350 µl of deionized water or PBS to resuspend the cells

b. Cells grown in a monolayer in a 96-well, 24-well, 12-well or 6-well plate:

1b. Discard as much of the supernatant as possible, then add 350 µl of PBS into each well. Let the plate stand for 1 minute at room temperature.

2b. Pipette up and down several times, and then transfer 350 µl of the cell lysate to a 2 ml microfuge tube (provided), if the volume of the homogenate is less than 350 µl, make it up to 350 µl with PBS.

c. Lymphocytes:

1c. Suspend the lymphocytes in 350 µl of PBS. Let the plate stand for 1 minute at room temperature.

2c. Pipette up and down several times, and then transfer 350 µl of the lymphocytes to a 2 ml microfuge tube (provided), if the volume of the homogenate is less than 350 µl, make it up to 350 µl with PBS.

d. Bone marrow:

1d. To obtain bone marrow samples, remove the femur and cut through the bone at both ends. Using a syringe and small gauge needle, inject 350 µl of PBS through one end of the bone and flush the marrow.

2d. Pipette up and down several times, and then transfer 350 µl of the marrow to a 2 ml microfuge tube (provided), if the volume of the homogenate is less than 350 µl, make it up to 350 µl with PBS.

e. Dried Blood Spot:

1e. Add 350 µl of PBS to dried blood spot. Let the plate stand for 1 minute at room temperature.

2e. Pipette up and down several times, and then transfer 350 µl of the blood mixture to a 2 ml microfuge tube (provided), if the volume of the homogenate is less than 350 µl, make it up to 350 µl with PBS.

f. Bone:

1f. Select 10-50 mg of bone, flash-freeze the bone sample by immersion in liquid nitrogen. Transfer the frozen bone to a mortar and pestle and use the pestle to pulverize it quickly and

vigorously until it is reduced to a fine powder. The pulverized bone add 350 µl of PBS, grind hard for 30 seconds.

Note: Maintaining the bone in a frozen state will improve grinding.

2f. Pipette up and down several times, and then pulverized bone 350 µl of the cell lysate to a 2 ml microfuge tube (provided), if the volume of the homogenate is less than 350 µl, make it up to 350 µl with PBS.

3. Add 0.8µl RNase A and mix immediately by vortexing for 15sce, let for 1 minute at room temperature.
4. Add 8 µl Proteinase K and 150 µl Buffer C-L. Mix immediately by vortexing for 1 minute. Briefly centrifuge to remove drops from inside the lid and incubate at 56°C for 10 minutes.

   * **Note**: Do not add Proteinase K directly to buffer C-L.
5. Add 350 µl Buffer P-D to the sample and mix by vortexing at top speed for 30 seconds. Centrifuge for 10 minutes at 12,000xg at ambient temperature to pellet cellular debris.

D. Purification of Genomic DNA from Yeast

1. Collect $2x10^6$-$5x10^7$ yeast cells and centrifuge for 1 minute at 10,000xg to pellet the cells. Resuspend the yeast cells in 350 µl of PBS and transfer to a mortar.Gradually add liquid nitrogen until the yeast suspension is completely frozen. Using the pestle, quickly and forcefully reduce it to a fine powder. Add liquid nitrogen to prevent the sample from thawing during pulverization. After grinding is complete, warm the mortar at 56°C in water bath until it just begins to melt.

   ***Note:** For yeast, an OD600 = 1 is approximately 3×10^7 cells/ml.

   ***Note:** Yeast cell walls are relatively tough, and the grinding time and frequency of grinding should be appropriately extended to ensure that the yeast cell walls are fully broken.

   ***Note:** Add liquid nitrogen as required to prevent the material from thawing during pulverization.
2. Add 1.2 µl of RNase A. Quickly and vigorously the sample for 30 seconds.
3. Transfer 350 µl of the yeast homogenate to a 2 ml microfuge tube (provided). If the volume of the homogenate is less than 350 µl, add additional PBS up to 350 µl.
4. Add 150 µl of Buffer C-L and 20 µl Proteinase K. Quickly grind the sample for 1min. Briefly centrifuge to remove drops from inside the lid and incubate at 56°C for 10 minutes.

   ***Note:** Do not add Proteinase K directly to buffer C-L.
5. Add 350 µl Buffer P-D to the sample and mix by vortexing at top speed for 30 seconds. Centrifuge for 10 minutes at 12,000xg at ambient temperature.

Follow steps 6-10 with optional vacuum or centrifugation protocol.

A. Vacuum protocol

6A. Attach the vacuum manifold base to a vacuum source. Firmly position the Miniprep column(s) into the complementary fittings on the manifold top. Transfer the clarified supernatant

obtained from Step 5 to the Miniprep column. Turn on the vacuum source and adjust to 20-30 inches Hg. Continue to apply the vacuum until no solution remains in the Miniprep column.

7A. Add 500 µl of Buffer W1 and draw all of the solution through the Miniprep column.

8A. Add 700 µl of Buffer W2 along the wall of Miniprep column to wash off residual Buffer W1 and draw all of the solution through the Miniprep column. Repeat this wash step with a second 700 µl aliquot of Buffer W2.

***Note**: Make sure that ethanol has been added into Buffer W2 concentrate.

***Note**: Add Buffer W2 along the tube wall to wash off any residual salt.

***Note**: Two washes with Buffer W2 are used to ensure the complete removal of salt, eliminating potential problems in subsequent enzymatic reactions.

9A. Transfer the Miniprep column to a 2 ml microfuge tube and centrifuge for 1 minute at 12,000xg.

B. Centrifuge protocol

6B. Place a Miniprep column into a 2 ml microfuge tube. Pipette the clarified supernatant obtained from step 5 into the Miniprep column. Centrifuge for 1 minute at 12,000xg.

7B. Discard the filtrate from the 2 ml microfuge tube. Place the Miniprep column back into the 2 ml microfuge tube. Pipette 500 µl of Buffer W1 to the Miniprep column and centrifuge at 12,000xg for 1 minute.

8B. Discard the filtrate and place the Miniprep column back into the 2 ml microfuge tube. Add 700 µl of Buffer W2 and centrifuge for 1 minute at 12,000xg. Discard the filtrate from the 2 ml microfuge tube and repeat this wash step with a second 700 µl aliquot of Buffer W2.

***Note**: Make sure that ethanol has been added into Buffer W2 concentrate.

***Note**: Two washes with Buffer W2 are used to ensure the complete removal of salt, eliminating potential problems in subsequent enzymatic reactions.

9B. Discard filtrate from the 2 ml microfuge tube. Place the Miniprep column back into the 2 ml microfuge tube and centrifuge for 1 minute at 12,000xg.

10. Transfer the Miniprep column into a clean 1.5 ml microfuge tube . To elute the genomic DNA, add 100-200 µl of Eluent (or deionized water) to the center of the membrane. Let it stand for 1 minute at room temperature. Centrifuge for 1 minute at 12,000xg.

***Note:** Pre-warming deionized water or Eluent at 65°C will often improve elution efficiency.

5. Overview

Add 350 µl of PBS
Add RNase A according to relevant protocols

Starting material

Add Proteinase K according to relevant protocols
Add 150 µl of Buffer C-L Incubate at 56°C for 15 minutes

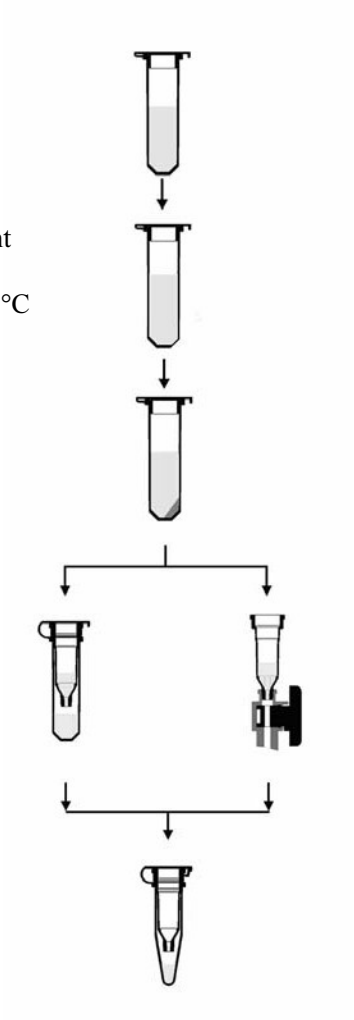
Proteinase K digest

Add 350 µl of Buffer P-D

Protein precipitation

Add 500 µl of Buffer W1
Add 700 µl of Buffer W2
Add 700 µl of Buffer W2

Binding
Washing



Add 100-200 µl of Eluent

Elution