WizPrep™ gDNA Mini Kit (Cell/Tissue)

- W71060-100 **100 prep**
- W71060-300 **300 prep**

Description

The WizPrep™ gDNA Mini Kit (Cell/Tissue) provides a fast and simple method to isolate genomic DNA from various animal tissue, cultured cells and bacteria.
The WizPrep™ gDNA Mini Kit (Cell/Tissue) uses silica-membrane technology to eliminate the cumbersome steps associated with loose resins or slurries. The kit is ready for use and can purify the genomic DNA from a wide variety of animal cell and tissue samples, and the whole process is completed in less than 20 minutes.
Purified DNA is suitable for PCR, restriction endonuclease digestion and Southern Hybridization.

Kit Contents

Contents	50 prep	100 prep	300 prep	Storage
GT1 Buffer	12 ml	25 ml	80 ml	R/T
GT2 Buffer	12 ml	25 ml	80 ml	R/T
W1 Buffer	28 ml	55 ml	180 ml	R/T
W2 Buffer(1)	8 ml	16 ml	50 ml	R/T
Elution Buffer	5 ml	10 ml	30 ml	R/T
Proteinase K**	22 mg	44 mg	132mg	4℃
Spin Columns*	50	100	300	R/T
Collection Tubes	50	100	300	R/T
Instruction Manual	1	1	1	R/T

(1) : Add absolute ethanol to the W2 Buffer prior to initial use (see the bottle label for volume).
* All Spin Columns are sterilized by electron beam.
** After receiving the Proteinase K, please store at 4℃.
After dissolved in distilled water, store the Proteinase K solution at 4℃ for up to 3 months. For longer storage (up to 1 year), the Proteinase K solution should be divided into small aliquot and stored at -20℃.
• R/T : Room temperature

Reagents and equipment to be supplied by user

- 96~100% ethanol (to prepare W2 Buffer)
- 1.5ml microcentrifuge tubes
- Sterile RNase-free pipette tips and Manual pipettors
- Centrifuge for microcentrifuge tubes
- Equipment for sample disruption and homogenization
- Personal protection equipment (lab coat, gloves, goggles)

Kit specifications

Parameter	Characteristics
Format	Silca-membrane spin column
Sample materials	< 25 mg tissue, 10^2 ~ 10^6 cultured cells
Typical yield	10~35 μg (depending on sample)
Elution volume	50~100 μl
Preparation time	< 20 minutes

Quality Control Analysis

The kit was qualified by isolating genomic DNA from 20mg of animal tissue and Gram-negative bacterial cell following the protocols outlined in the manual.

Quality Authorized by : Jamie Ahn

Protocol

Before starting ;
1) Add absolute ethanol to the W2 Buffer prior to initial use (see the bottle label for volume).
2) Dissolve each vial of Proteinase K (22mg) in 1,100μl of D.W
3) If a precipitate has formed in GT1 Buffer, dissolve by incubating at 56°C before use.

A. Cultured cells from tissue (< 5 X 10^6)

- Harvest the cells and transfer to 1.5ml tube.
- Centrifuge for 10 seconds at 13,000 rpm. to pellet the cells and carefully remove the supernatant.
- Add **200μl of PBS or TE buffer** to the pellet and completely resuspend the pellet by vortexing or pipetting.
  Proceed to Step 2.

B. Animal tissue (< 25 mg), for spleen (< 10 mg)

- Cut up to 20 mg of animal tissue (or 0.5 cm of mouse tail) then transfer it to a 1.5 ml tube.

NOTE: If tissue has a higher number of cells (e.g. spleen or liver), reduce the starting material to 10 mg.

- Add **200μl of GT1 Buffer** to the tube and homogenize the sample tissue by grinding. Proceed to Step 2.

C. Bacteria (Gram-negative)

- Centrifuge cells (<2x10^9) for 10 min. at 5,000 x g (7,500 rpm).
- Carefully remove the supernatant and resuspend bacterial pellet in **180μl of GT1 Buffer**.
  Proceed to Step 2.

Step 2 : Lysis step

- Add **200μl of GT2 Buffe**r and **20μl of Proteinse K** to the sample and mix by vortexing.
- Incubate at 56℃ for 10 minutes. During incubation, invert the tube every 5 minutes.

Step 3: Binding step

- Add **200μl of 100% ethanol** to the sample lysates and mix by vortexing briefly.
- Apply the mixture to the Spin Column and centrifuge for 1 min. at 13,000 rpm.
- Discard the flow-through and re-connect with the Spin Column.

Step 3 : Wash Step

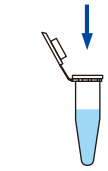
- Add **500μl of W1 Buffer** to the Spin Column and centrifuge for 1 min. at 13,000 rpm.
- Discard the flow-through and re-connect with Spin Column.
- Add **700μl of W2 Buffer** (ethanol added) in the Spin Column and centrifuge for 1 min. at 13,000 rpm.
- Discard the flow-through and re-connect the Spin Column and centrifuge for 2 min. at 13,000 rpm.

Step 4 : Elution Step

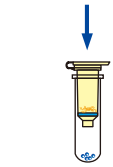
- Connect the Spin Column and new 1.5 ml tube.
- Add **50~100μl of Elution Buffer** and incubate at R/T for 1 min.
- Centrifuge for 1 min. at 13,000 rpm.
- Discard the Spin Column and eluted purified DNA for use next step.
- Eluted DNA are stored at –20°C for a few days, –70°C for long term storage.

Quick Protocol


Tissue (20mg)
GT1 Buffer (200μl)
Homogenization


Proteinase K (20μl)
GT2 Buffer (200μl)
56℃ / 10 min.


100% EtOH (200μl)


W1 Buffer (500μl)


W2 Buffer (700μl)


Elution Buffer (50μl)

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Troubleshooting

Problem	Possible Cause	Solution and Explanation
The Spin column is clogged	The sample is too large	Do not exceed the recommended amount of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. Clogging can also be alleviated by increasing the g-force and/or centrifuging for a longer period of time until the lysate passes through the column.
The lysate is very gelatinous prior to loading onto the column	The lysate solution mixture is not homogeneous	To ensure a homogeneous solution, vortex for 10~15 seconds before applying the lysate to the spin column.
	Maximum number of cells or amount of issue exceeds kit specifications	Refer to specifications to determine if amount of starting material falls within kit specifications.
The yield of genomic DNA is low	Improper storage of samples	Tissue samples and cell pellets may be frozen and stored at -20°C or -70°C. Repeated freezing and thawing of stored samples should be avoided, as this may lead to decreased yields of DNA.
	Incomplete lysis of cells	Ensure efficient homogenization of tissue samples and extend the incubation time of Proteinase K digestion or reduce the amount of tissue or cells used for lysis.
The genomic DNA is sheared	The genomic DNA was handled improperly	Pipetting steps should be handled as gently as possible. Reduce vortexing times during mixing steps (no more than 10-15 seconds).
	Improper storage of sample	Repeated freezing and thawing of stored samples should be avoided as this may lead to decreased DNA size.
	The sample is old	Sheared DNA may be obtained from old tissue or cell samples. Fresh samples are recommended for maximum genomic DNA yield
DNA does not perform well in downstream applications.	DNA was not washed with the provided W2 Buffer	Ensure the column was washed once with W1 Buffer and twice with W2 Buffer.
	Ethanol carryover	Ensure that the dry spin under the Column Wash Step is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.