LITE Genomic DNA Kit – Tissue

#GK03Lite (200 preps) | GK03s (trial size, 4 preps)
(FOR RESEARCH ONLY)

Sample : up to 30mg of tissue or 25mg of paraffin-embedded tissue (FFPE)
Expected Yield : up to 50µg DNA
Format : spin column
Operation Time : within 30 minutes (60 minutes for FFPE)
Elution Volume : 30-200µl

Product: The LITE Genomic DNA Kit – Tissue - provides an efficient and fast method for the purification and or concentration of high-quality total DNA (including genomic DNA, mitochondrial DNA and viral DNA) from a variety of tissues, including tailsnips, liver, kidney, brain, adipose tissue, earpunches, insects, and FFPE. Eluted purified DNA (approximately 20-30kb) is suitable for PCR, and other enzymatic reactions.

The LITE Genomic DNA Kit – Tissue - uses chaotropic salts and proteinase K to lyse cells and to denature proteins. The provided micropestle improves tissue sample homogenization and therefore to reduce the time required for cell lysis. The buffer system is optimized to allow selective binding of DNA to the glass fiber matrix of the spin column[1]. Contaminants such as proteins, divalent cations, unincorporated nucleotides, and enzyme inhibitors are completely removed using a Wash Buffer (containing ethanol) in a simple centrifugation step. The purified genomic DNA is subsequently eluted by a low salt Elution Buffer or TE or water. The entire procedure can be completed within an hour without phenol/chloroform extraction or alcohol precipitation, with typical DNA yields of up to 50 µg.

QC: The quality of the LITE Genomic DNA Kit – Tissue - is tested on a lot-to-lot basis by isolating total DNA from 20mg of a mouse liver sample. Purified DNA is quantified using a spectrophotometer with a typical yield of more than 10µg of genomic DNA and a A260nm/A280nm ratio of 1.8-2.0. Quality is further checked by agarose gel electrophoresis.

Caution: Buffer TC1 contains guanidine hydrochloride which is a harmful irritant. During operation, always wear a lab coat, disposable gloves, and protective goggles.

References:

1. Vogelstein, B., and Gillespie, D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 615-619

Kit Contents	LITE (200 preps)	sample (4 preps)	GRS* (100 preps)
Buffer BC2	60 ml	3 ml	30 ml
Buffer TC1	80 ml	4 ml	40 ml
Wash Buffer 1	90 ml	2 ml	45 ml
Wash Buffer 2**	50 ml	1 ml	25 ml
Elution Buffer	60 ml	1 ml	30 ml
RNase A (10mg/ml)	-	-	0.55ml
Proteinase K***	4x 11mg	1mg	2x 11mg
gDNA plus mini spin column	200	4	100
1.5-ml microtube (DNase/RNase free)	-	-	200
2-ml collection tube	400	8	200
micropestle	200	4	100

Required Components (not included)
Ethanol (96%-100%)
Centrifuge for microtubes
Pipets and tips
Vortex
Water bath or Thermoblock
Xylene
15-ml centrifuge tubes
1.5-ml microtube (DNase/RNase free)
RNase A

* GRS versions of NAP Kits contain all the necessary components for the standard protocols, including optional steps.

** Add Ethanol (96%-100%) [not included] to Wash Buffer 2, as indicated on the bottle, prior to initial use. After Ethanol has been added, mark the bottle to indicate that this step has been completed. Close bottle tightly to avoid ethanol evaporation.

*** Add Water (ultrapure) [not included] to Proteinase K, as indicated on the tube, prior to initial use. After Water has been added, mark the tube to indicate that this step has been completed.

Storage: RNase A and Proteinase K are stable enzymes and transport is carried out either with or without cooling. Upon arrival, RNase A (10mg/ml) should be stored at -20°C and proteinase K (powder) at 4°C. Once water has been added, it is recommended to store the Proteinase K solution at -20°C. All other components should be stored at room temperature. Examine solutions for precipitates before use. Any precipitate may be re-dissolved by warming the solution to 37°C followed by cooling to 25°C. Store for up to 2 years.

FFPE TISSUE DEPARAFFINIZATION

Prior to DNA purification, FFPE tissue needs to be deparaffinized. One method is by using Xylene (not included). Other methods may also be used, but have not been tested with this kit.

1. Slice small sections (up to 25mg) from blocks of paraffin-embedded tissue and transfer to a 1.5-ml microcentrifuge tube. Add 1 ml of Xylene. Shake vigorously, and incubate at room temperature for 10 minutes. During incubation, invert the tube regularly.

2. Centrifuge for 3 minutes at 14,000g-16,000g and discard the supernatant, using a pipet.

3. Add 1 ml of absolute ethanol to the pellet and mix by inverting. Centrifuge for 3 minutes at 14,000g-16,000g and discard the supernatant, using a pipet. Repeat this step twice.

4. Open the tube and air-dry (37°C) for 15 minutes to evaporate any remaining ethanol.

5. Proceed with step 2 of the Tissue Protocol on page 3 (Addition of Buffer BC2).

PROTOCOL FOR DNA PURIFICATION FROM TISSUE

1) Cut up to 30 mg of animal tissue (or 5 mm of mouse tail) and transfer to a 1.5-ml microcentrifuge tube. In case of some tissues, like spleen that contain a very high number of cells, the starting material should be reduced to 10 mg. Use the micropestle to grind the tissue to pulp.

2) Add 200 µl of Buffer BC2 and continue to homogenize the sample by grinding.

3) Add 20 µl of Proteinase K (10 mg/ml), mix by shaking vigorously, and incubate at 60ºC for 30 minutes. During incubation, invert the tube regularly.

4) Add 200 µl of Buffer TC1, mix by shaking vigorously, and incubate at 60ºC for at least 20 minutes. During incubation, invert the tube regularly. (Note that sample lysate should become clear. If there is still insoluble material present following the lysis step, centrifuge for 2 minutes at 14,000g-16,000g and transfer the supernatant to a new 1.5-ml microcentrifuge tube). [At this time, preheat the Elution Buffer in a 60ºC water bath to be used in step 11].

5) [**Optional step; If RNA-free DNA is required**] Allow the mixture to cool to room temperature and add 4µl of RNase A (10 mg/ml), vortex, and incubate for 5 minutes at room temperature.

6) Add 200 µl of absolute ethanol to the lysate and mix immediately by shaking vigorously for 10 seconds. In case precipitate appears, break it up by pipetting.

7) Place the gDNA plus mini spin column in a 2-ml collection tube and transfer the sample mixture (including any precipitate if present) to the column.

8) Centrifuge at 14,000g-16,000g for 2 minutes. Discard the collection tube containing the flow-through and place the gDNA plus mini spin column in a new collection tube.

9) Add 400 µl of Wash Buffer 1 and centrifuge at 14,000g-16,000g for 30 seconds. Discard the flow-through and place the gDNA plus mini spin column back in the collection tube. Add 600 µl of Wash Buffer 2* and centrifuge at 14,000g-16,000g for 30 seconds (*Ensure ethanol was added 1st time prior to use).

10) Discard the flow-through and place the gDNA plus mini spin column back in the collection tube and centrifuge for another 3 minutes at 14,000g-16,000g to dry the matrix of the column.

11) Transfer the spin column to a new 1.5-ml microcentrifuge tube and pipet 100 µl preheated Elution Buffer directly to the center of the spin column without touching the membrane. Incubate at room temperature 5 minutes.
Notes: 1) Instead of Elution Buffer, DNA can also be diluted with TE or water; pH ideally should be 8.0-8.5. 2) Standard elution volume is 100µl. To increase concentration, elute with 30-50µl. To increase yield, elute with 200µl.

12) Centrifuge for 30 seconds at 14,000g-16,000g to elute purified genomic DNA. Discard the spin column and use DNA immediately or store at -20ºC.

TROUBLESHOOTING

1. **Low Yield**
    - *Clogged Column*
        - i. Reduce the amount of sample material
        - ii. Prior to loading the column, break up precipitate in ethanol-added lysate
    - *Incomplete tissue lysis*
        - i. Add additional Proteinase K and extend the incubation time in the Lysis step
        - ii. Prior Following lysis step, centrifuge for 2 minutes at 14,000g-16,000g to remove debris. Transfer the supernatant to a new microcentrifuge tube and proceed with the DNA binding step
    - *Incorrect DNA Elution Step*
        - i. Ensure that the Elution Buffer is completely adsorbed after being added to the center of the spin column
    - *Incomplete DNA Elution*
        - i. Elute twice to increase overall yield

2. **Low Quality**
    - *Low performance in downstream applications*
        - i. Residual ethanol contamination interferes with downstream applications. Following the wash step, dry the spin column with additional centrifugation for 5 minutes or incubation at 60°C for 5 minutes in order to evaporate ethanol.
        - ii. In case of protein contamination, the amount of starting material should be reduced.
        - iii. DNA denaturation/fragmentation (which can be detected by gel analysis), may be the result of improper/long storage of "fresh" samples. Use fresh samples, or freeze samples in liquid nitrogen immediately and store at -80°C

DNA PURIFICATION OTHER SAMPLE TYPES

This kit can also be used, with some minor adaptations, for the purification of DNA from Amniotic Fluid, Buccal Swab, Stool, Bone and Tooth, and Soil. These protocols can be downloaded from our website.