LITE Genomic DNA Kit – BroadRange

#GK06Lite (200 preps) | GK06s (trial size, 4 preps)
(FOR RESEARCH ONLY)

Sample : up to 200µl of whole blood (fresh or frozen), plasma, serum, buffy coat, body fluids, up to 25mg of tissue, up to 25mg of paraffin-embedded tissue (FFPE), up to 15ml of amniotic fluid, cultured animal cells, buccal swab
Expected Yield : 5µg DNA (in case of whole fresh blood)
Format : spin column
Operation Time : depending on sample type
Elution Volume : 30-200µl

Product: The LITE Genomic DNA Kit – BroadRange - provides an efficient and fast method for the purification of high-quality total DNA (including genomic DNA, mitochondrial DNA and viral DNA) from a broad range of samples, including whole and frozen blood, plasma, serum, body fluids, amniotic fluid, buccal swab, cultured cells, tissue and FFPE. Eluted purified DNA (approximately 20-30kb) is suitable for PCR, and other enzymatic reactions.

The LITE Genomic DNA Kit – BroadRange - uses Proteinase K and chaotropic salts to lyse cells and to denature proteins. 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, 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 30 minutes without phenol/chloroform extraction or alcohol precipitation, with typical DNA yields of 5 µg from 200µl of whole human fresh blood.

QC: The quality of the LITE Genomic DNA Kit – BroadRange - is tested on a lot-to-lot basis by isolating total DNA from 200µl of whole human blood. Purified DNA quantified using a spectrophotometer with a typical yield of 4-6µg of genomic DNA with a A260nm/A280nm ratio of 1.8-2.0) and further checked by agarose gel electrophoresis.

Caution: Buffer BR2 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 BR1	60 ml	3 ml	30 ml
Buffer BR2	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 spin column	200	4	100
1.5-ml microtube (DNase/RNase free)	-	-	200
2-ml collection tube	400	8	200

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

* 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.

**** PBS (Phosphate Buffered Saline) = aqueous solution of 137mM NaCl, 2.7mM KCl,10mM Na_2HPO_4, and 2.0mM KH_2PO_4 pH7.4

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.

MAIN PROTOCOL FOR DNA PURIFICATION
[From whole blood (fresh or frozen), as well as for serum, plasma, buffy coat or body fluids]

1) Transfer 50 to 200µl of sample (in the case of blood fresh or from a blood collection tube containing EDTA or other anticoagulant), to a 1.5-ml microcentrifuge tube. Adjust volume to 200µl with PBS, and add 20µl of Proteinase K (10mg/ml) to the sample. Mix by pipetting. Incubate at 60ºC for 5 minutes.

2) Add 200µl of Buffer BR2 and vortex. Incubate at 60ºC for 5 minutes. During incubation invert the tube regularly. [At this time, preheat the Elution Buffer in a 60ºC water bath to be used in step 9].

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

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

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

6) Centrifuge at 14,000g-16,000g for 1 minute. If the mixture did not flow-through the membrane completely, increase centrifugation time. Discard the collection tube containing the flow-through and place the spin column in a new collection tube.

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

8) Discard the flow-through and place the 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.

9) Transfer the spin column to a new 1.5-ml microcentrifuge tube and pipet 100µl preheated Elution Buffer directly to the canter of the spin column without touching the membrane. Incubate at room temperature for 3-5 minutes (in case of amniotic fluid protocol: incubate at 37ºC for 5-10 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.

10) 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.

PARAFIN-EMBEDDED 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 up to 25 mg from blocks of FFPE tissue and transfer to a 1.5-ml microcentrifuge tube.

2) Add 1 ml of Xylene and mix by shaking vigorously

3) Incubate at room temperature for 10 minutes. During incubation, invert tube regularly. Centrifuge at 14,000g-16,000g for 3 minutes. Discard supernatant.

4) Add 1 ml of absolute ethanol and mix by inverting ten times. Centrifuge at 14,000g-16,000g for 3 minutes. Discard supernatant.

5) Repeat step 4. After removing the supernatant, open the tube and incubate at 37°C for 15-20 minutes to evaporate any remaining ethanol.

6) Proceed with step 2 of the Tissue protocol (see hereunder)

PROTOCOL FOR DNA PURIFICATION FROM TISSUE

1) Cup to 25 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. Using a micropestle to grind the tissue to pulp will increase yield.

2) Add 200µl of Buffer BR1 and 20µl of Proteinase K (10mg/ml). Mix by vortexing. Incubate at 60°C overnight (or until lysate becomes clear). Occasional or continues shaking is recommended.

3) Add 200µl of Buffer BR2. Mix by shaking vigorously. If insoluble material is present following incubation, centrifuge at 14,000g-16,000g for 2 minutes and transfer the supernatant to a new 1.5-ml microcentrifuge tube [At this time, preheat the Elution Buffer in a 60°C water bath].

4) Continue with step 3 of the main protocol on page 3.

PROTOCOL FOR DNA PURIFICATION FROM BUCCAL SWAB

1) Add 500µl of Buffer BR1 and 20µl of Proteinase K (10mg/ml) to a 1.5-ml microcentrifuge tube, place the swab into the tube and incubate at 60ºC for 10 min.

2) Discard the swab and add 500µl of Buffer BR2. Immediately, mix by shaking vigorously and then incubate at 60ºC for another 10 min. [At this time, preheat the Elution Buffer in a 60ºC water bath to be used later].

3) Add 500µl of absolute ethanol to the lysate and mix immediately for 10 seconds by shaking vigorously. In case precipitate appears, break it up by pipetting.

4) Place the gDNA plus spin column in a 2-ml collection tube and transfer 700µl of the sample mixture (including any precipitate if present) to the column.

5) Centrifuge at 14,000g-16,000g for 1 minute. Discard the flow-through and repeat the binding step with the remaining mixture from step 3. After centrifugation, discard the collection tube containing the flow-through and place the column in a new 2-ml collection tube. Proceed with the wash step (step 7 of the main protocol on page 3).

PROTOCOL FOR DNA PURIFICATION FROM AMNIOTIC FLUID

1) Transfer approximately 10ml of amniotic fluid to a 15-ml centrifuge tube and centrifuge at 14,000g-16,000g for 3 minutes. Discard the supernatant

2) Resuspend the pellet with 200µl of Buffer BR1 and transfer the suspension to a 1.5-ml microcentrifuge tube.

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

4) Add 200µl of Buffer BR2 and mix by shaking vigorously. Incubate at 60ºC for 20 minutes or until lysate is clear. During incubation, invert the tube regularly [At this time, pre-heat the Elution Buffer to 60ºC].

5) Proceed with step 3 of the main protocol on page 3.

PROTOCOL FOR DNA PURIFICATION FROM CULTURED ANIMAL CELLS

(In case of using adherent cells, trypsinize cells before harvesting)

1) Transfer up to $1x10^7$ cells to a 1.5-ml microcentrifuge tube.
2) Centrifuge for 5 minutes at 300g
3) Discard supernatant and resuspend cells in 200µl of PBS
4) Add 20µl of Proteinase K (10mg/ml) to the sample. Mix by pipetting.
5) Incubate at 60°C for 5 minutes and continue with cell lysis (step 3 of the main protocol on page 3).

TROUBLESHOOTING

1. **Low Yield**
    - *Clogged Column*
        - **i.** Reduce the amount of sample material
    - *Precipitate was formed at DNA Binding Step*
        - **i.** Reduce the amount of sample material
        - **ii.** Prior to loading the column, break up precipitate in ethanol-added lysate
    - *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.** DNA denaturation/fragmentation (which can be detected by gel analysis), may be the result of improper/long storage of the sample.