LITE Genomic DNA Kit – Blood & Cultured Cells

#GK02Lite (200 preps) | GK02s (trial size, 4 preps)
(FOR RESEARCH ONLY)

Sample : up to 300µl of whole blood, up to 200µl of frozen blood, up to 200µl of buffy coat, up to $1x10^7$ cultured animal cells.
Expected Yield : up to 50µg DNA
Format : spin column
Operation Time : within 60 minutes
Elution Volume : 30-200µl

Product: The LITE Genomic DNA Kit – Blood & Cultured Cells - 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 samples, including whole and frozen blood, buffy coat and cultured animal cells. Eluted purified DNA (approximately 20-30kb) is suitable for PCR, and other enzymatic reactions.

The LITE Genomic DNA Kit – Blood & Cultured Cells - uses 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, 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 6µg from 200µl of whole human blood or up to 50µg from 200µl of buffy coat.

QC: The quality of the LITE Genomic DNA Kit – Blood & Cultured Cells - 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 an A260nm/A280nm ratio of 1.6-1.8, and further ensured by agarose gel electrophoresis.

Caution: Buffer BC1 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)
Red Blood Cell Lysis Buffer	270 ml	6 ml	135 ml
Buffer BC1	80 ml	2 ml	40 ml
Buffer BC2	60 ml	1.5 ml	30 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**	-	-	3x11mg
Genomic DNA mini 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****
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.

PROTOCOL FOR DNA PURIFICATION FROM FRESH BLOOD

[Red Blood Cell Lysis Buffer Treatment removes non-nucleated red blood cells and reduces hemoglobin contamination. If the blood sample is less than 5µl or if the sample consists of nucleated blood cells (e.g., avian blood), it is recommended to use the Cultured Cells Protocol (page 6) to purify genomic DNA].

1) Transfer up to 300µl of fresh blood (from a blood collection tube containing EDTA or other anticoagulant) to a 1.5-ml microcentrifuge tube, and add 3 volumes of Red Blood Cell Lysis Buffer. Mix by inversion. DO NOT VORTEX! (e.g., add 600µl of the lysis buffer to 200µl of fresh blood).

2) Incubate at room temperature for 10 minutes.

3) Centrifuge for 5 minutes at 3,000g and remove the supernatant completely. Resuspend the pellet with 100µl of Red Blood Cell Lysis Buffer and add 200µl of Buffer BC1. Mix well by shaking vigorously.

4) Incubate during 10 minutes at 65ºC-70ºC or until the sample lysate is clear. During incubation, invert the tube regularly [At this time, preheat the Elution Buffer in a 70ºC water bath to be used in step 11].

5) **[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.

6) 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.

7) Place the genomic DNA 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 5 minutes. Discard the collection tube containing the flow-through and place the genomic DNA mini spin column in a new collection tube.

9) Add 400µl of Wash Buffer 1 and centrifuge at 14,000g-16,000g for 30 seconds. Discard the flow-through and place the genomic DNA mini 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).

10) Discard the flow-through and place the genomic DNA 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 100µl preheated Elution Buffer directly to the center of the spin column without touching the membrane. Incubate at room temperature for 3-5 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.

PROTOCOL FOR DNA PURIFICATION FROM FROZEN BLOOD

1) Transfer up to 200µl of blood (if the sample volume is less than 200µl, add PBS to a final volume of 200µl) to a 1.5-ml microcentrifuge tube, and add 30µl of Proteinase K (10mg/ml). Mix by inversion.

2) Incubate at 60°C for 15 minutes.

3) Add 200µl of Buffer BC1. Mix by shaking vigorously, and incubate at 70°C for 15 minutes. During incubation, invert the tube regularly [At this time, preheat the Elution Buffer in a 70°C water bath to be used in step 11].

4) **[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.

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

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

7) Centrifuge at 14,000g-16,000g for 5 minutes. Discard the collection tube containing the flow-through and place the genomic DNA mini spin column in a new collection tube.

8) Add 400µl of Wash Buffer 1 and centrifuge at 14,000g-16,000g for 30 seconds. Discard the flow-through and place the genomic DNA mini spin column back in the collection tube.

9) 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).

10) Discard the flow-through and place the genomic DNA 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 100µl preheated Elution Buffer directly to the center of the spin column without touching the membrane. Incubate at room temperature for 3-5 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 -20°C.

PROTOCOL FOR DNA PURIFICATION FROM BUFFY COAT

[Red Blood Cell Lysis Buffer Treatment removes non-nucleated red blood cells and reduces hemoglobin contamination. If the blood sample is less than 5µl or if the sample consists of nucleated blood cells (e.g., avian blood), it is recommended to use the Cultured Cells Protocol (page 6) to purify genomic DNA].

1) Transfer up to 200µl of buffy coat to a 1.5-ml microcentrifuge tube, and add 3 volumes of Red Blood Cells Lysis Buffer. Mix by inversion and incubate for 10 minutes at room temperature. During incubation, invert the tube regularly (e.g., add 600µl of the red blood cell lysis buffer to 200µl of buffy coat).

2) Centrifuge for 1 minute at 14,000g-16,000g and remove the supernatant completely. Resuspend the white pellet with 500µl of Red Blood Cell Lysis Buffer.

3) Centrifuge for 1 minute at 14,000g-16,000g and remove the supernatant completely. Resuspend the white pellet with 200µl of Red Blood Cell Lysis Buffer and add 250µl of Buffer BC1. Mix well by shaking vigorously.

4) Incubate at 65ºC-70ºC for 30 minutes or until the sample lysate is clear. During incubation, invert the tube regularly [At this time, preheat the Elution Buffer in a 70ºC water bath to be used in step 11].

5) **[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.

6) Add 250µl 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 genomic DNA 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 5 minutes. Discard the collection tube containing the flow-through and place the genomic DNA mini spin column in a new collection tube.

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

10) Discard the flow-through and place the genomic DNA 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 100µl preheated Elution Buffer directly to the center of the spin column without touching the membrane. Incubate at room temperature for 3-5 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.

PROTOCOL FOR DNA PURIFICATION FROM CULTURED CELLS

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

1)
   a) Transfer up to 1x10^7 cells to a 1.5-ml microcentrifuge tube, and harvest by centrifugation for 20 seconds at 6,000g. Discard the supernatant and add 150µl Red Blood Cell Lysis Buffer. Mix by inversion.

   b) In case of fresh blood (except human blood), add 150µl of Buffer BC2, and mix by shaking vigorously.
      i) For mammalian blood sample (non-nucleated), the sample can be up to 50µl
      ii) For nucleated erythrocytes (e.g. fish or birds), the sample can be up to 10µl

2) Add 200µl of Buffer BC1, mix by shaking vigorously for 5 seconds, and incubate at 70°C for 10 minutes or sample lysate is clear. During incubation, invert the tube regularly [At this time, preheat the Elution Buffer in a 70°C 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 immediately by shaking vigorously for 10 seconds. In case precipitate appears, break it up by pipetting.

5) Place the genomic DNA mini 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 2 minutes. Discard the collection tube containing the flow-through and place the genomic DNA mini 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 genomic DNA mini 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 genomic DNA 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.

9) Transfer the spin column to a new 1.5-ml microcentrifuge tube and pipet 100µl preheated Elution Buffer directly to the center of the spin column without touching the membrane. Incubate at room temperature for 3-5 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.

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 -20°C.

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 60ºC 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 "fresh" blood.