GRISP RESEARCH SOLUTIONS

LITE PCR & Gel Band Purification Kit

#GK01Lite (300 preps) | GK01s (trial size, 4 preps)
(FOR RESEARCH ONLY)

Sample : up to 100µl of PCR products or DNA fragments from enzymatic reactions, up to 300mg of agarose gel
Expected Yield : typical recoveries up to 95% (PCR Clean-up) or up to 90% (Gel Extraction)
Format : spin column
Operation Time : 10 minutes (PCR clean-up), 20 minutes (Gel Extraction)
Elution Volume : 20-50µl

Product: The LITE PCR & Gel Band Purification Kit provides an efficient and fast method for the purification and or concentration of high-quality DNA fragments (70bp to 15kb) from PCR reactions, enzymatic restriction digestion or from agarose gels. Eluted DNA is suitable for all common downstream applications including PCR, enzymatic restriction digestion, cloning, library construction, Southern blot analysis, and DNA sequencing.

The LITE PCR & Gel Band Purification Kit uses chaotropic salts to dissolve agarose gel and to denature enzymes such as restriction enzymes or polymerases. 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 in approximately 20 minutes without phenol/chloroform extraction or alcohol precipitation, with a typical DNA recovery of 80% to 90% for agarose gel extraction and up to 95% for PCR or Restriction Enzyme reactions.

QC: The quality of the LITE PCR & Gel Band Purification Kit is tested on a lot-to-lot basis by isolating DNA fragments from either PCR reactions or agarose gel followed by subsequent electrophoresis.

Caution: During operation, always wear a lab coat, disposable gloves, and protective goggles. The Gel Solubilization Solution contains guanidine thiocyanate which is a harmful irritant.

References:

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

Kit Contents	LITE (300 preps)	sample (4 preps)	GRS* (100 preps)
Gel Solubilization Solution	240 ml	3 ml	80 ml
3M Sodium Acetate pH 5.0	200 µl	-	200 µl
Wash Buffer 1	130 ml	2 ml	45 ml
Wash Buffer 2**	75 ml	1 ml	25 ml
Elution Buffer	30 ml	4	6 ml
DNA fragment mini spin Column	300	4	100
1.5-ml microtube (DNase/RNase free)	-	-	200
2-ml collection tube	300	4	100

Required Components (not included)
Ethanol (96%-100%)
Centrifuge for microtubes
Pipets and tips
Vortex
Water bath or Thermoblock
Scalpels/Razor blades or
band cutters
1.5-ml microtubes (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.

Storage: All 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 1 year.

Notes

pH Indicator

The Gel Solubilization Solution is premixed with a pH indicator to ensure optimal pH (solution should be yellowish). DNA binding is facilitated by pH <7.5 and if the pH exceeds the optimal pH, the colour of the solution appears purple. If this occurs, one can adjust the pH by adding 3M Sodium Acetate pH 5.0 (included) until colour becomes yellowish. Moreover, the pH indicator allows for easy observation of gel solubilization.

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PROTOCOL FOR GEL BAND PURIFICATION

1) Excise the agarose slice containing the DNA fragment of interest with a clean scalpel, razor blade or band cutter, and remove any extra agarose to minimize gel volume.

2) Transfer up to 300mg of the gel slice to a 1.5-ml microcentrifuge tube and add 500µl of the Gel Solubilization Solution and vortex briefly.

3) Incubate at 55ºC-60ºC for 10-15 minutes (or until the gel slice has been dissolved completely). During incubation, invert the tube regularly. After the gel has been dissolved, allow the sample mixture to cool to room temperature.

4) **[optional]** If the mixture has turned purple, add 10 µl of 3M Sodium Acetate pH 5.0 to adjust pH and mix thoroughly.

5) Place the DNA fragment mini spin column in a 2-ml collection tube and transfer up to 800µl of the sample mixture to the column.

6) Centrifuge at 14,000g-16,000g for 30 seconds.

7) Discard the collection tube containing the flow-through, and place the spin column back in the collection tube.

8) Add 400µl of Wash Buffer 1. Centrifuge for 14,000g-16,000g for 30 seconds and discard the flow-through. Place the column back into the collection tube.

9) Add 600µl of Wash Buffer 2* and let stand for 1 minute. (ensure ethanol was added 1st time prior to use).

10) Centrifuge at 14,000g-16,000g for 30 seconds and discard the flow-through

11) Place the spin column back in the collection tube and centrifuge at 14,000g-16,000g for another 3 minutes to dry the matrix of the column.

12) Transfer the spin column to a new 1.5-ml microcentrifuge tube and pipet 20µl-50µl of Elution Buffer directly to the center of the spin column without touching the membrane. Incubate at room temperature for 2 minutes.
Notes: 1) Yield could be increased using pre-warmed Elution Buffer (60ºC). 2) Instead of Elution Buffer, DNA can also be eluted with TE or water; pH ideally should be 8.0-8.5.

13) Centrifuge for 2 minutes at 14,000g-16,000g to elute purified DNA. Discard the spin column and use DNA immediately or store at -20ºC.

PROTOCOL FOR GEL BAND PURIFICATION (PROCEDURE FOR SEQUENCING)

1) Excise the agarose slice containing the DNA fragment of interest with a clean scalpel, razor blade or band cutter, and remove any extra agarose to minimize gel volume.

2) Transfer up to 300mg of the gel slice to a 1.5-ml microcentrifuge tube and add 500µl of the Gel Solubilization Solution and vortex briefly.

3) Incubate at 55ºC-60ºC for 10-15 minutes (or until the gel slice has been dissolved completely). During incubation, invert the tube regularly. After the gel has been dissolved, allow the sample mixture to cool to room temperature.

4) **[optional]** If the mixture has turned purple, add 10 µl of 3M Sodium Acetate pH 5.0 to adjust pH and mix thoroughly.

5) Place the DNA fragment mini spin column in a 2-ml collection tube and transfer up to 800µl of the sample mixture to the column.

6) Centrifuge at 14,000g-16,000g for 30 seconds.

7) Discard the collection tube containing the flow-through, and place the spin column back in the collection tube.

8) Add 600µl of Wash Buffer 2* and let stand for 1 minute (ensure ethanol was added 1st time prior to use).

9) Centrifuge for 14,000g-16,000g for 30 seconds and discard the flow-through. Place the column back into the collection tube.

10) Repeat steps 8 and 9 once.

11) Place the spin column back in the collection tube and centrifuge at 14,000g-16,000g for another 3 minutes to dry the matrix of the column.

12) Transfer the spin column to a new 1.5-ml microcentrifuge tube and pipet 20µl-50µl of Elution Buffer directly to the center of the spin column without touching the membrane. Incubate at room temperature for 2 minutes.
Notes: 1) Yield could be increased using pre-warmed Elution Buffer (60ºC). 2) Instead of Elution Buffer, DNA can also be eluted with TE or water; pH should be 8.0-8.5.

13) Centrifuge for 2 minutes at 14,000g-16,000g to elute purified DNA. Discard the spin column and use DNA immediately or store at -20ºC.

PROTOCOL FOR PCR CLEAN UP

1) Transfer up to 100µl of the PCR reaction solution to a 1.5-ml microcentrifuge tube. Add 5 volumes of Gel Solubilization Solution and mix by vortexing (e.g., 25µl PCR reaction volume plus 125µl gel solubilization solution).

2) **[optional]** If the mixture has turned purple, add 10 µl of 3M Sodium Acetate pH 5.0 to adjust pH and mix thoroughly.

3) Place the DNA fragment mini spin column in a 2-ml collection tube, and transfer the sample mixture to the column.

4) Centrifuge at 14,000g-16,000g for 30 seconds.

5) Discard the collection tube containing the flow-through, and place the spin column back in the collection tube.

6) Add 600µl Wash Buffer 2* and let stand for 1 minute (ensure ethanol was added 1st time prior to use).

7) Centrifuge at 14,000g-16,000g for 30 seconds and discard the flow-through

8) Place the spin column back in the collection tube and centrifuge at 14,000g-16,000g for another 3 minutes to dry the matrix of the column.

9) Transfer the spin column to a new 1.5ml microcentrifuge tube and pipet 20µl-50µl Elution Buffer directly to the center of the spin column without touching the membrane. Incubate at room temperature for 2 minutes.
   Notes: Yield could be increased using pre-warmed Elution Buffer (60ºC). Instead of Elution Buffer, DNA can also be eluted with TE or water; pH should be 8.0-8.5.

10) Centrifuge for 2 minutes at 14,000g-16,000g to elute purified DNA. Discard the spin column and use DNA immediately or store at -20ºC.

TROUBLESHOOTING

1. **Low Yield**
   - *Gel slice did not dissolve completely*
     - **i.** The gel slice was too big. If using >300mg of agarose, separate into multiple tubes
     - **ii.** Raise the incubation temperature to 60ºC and/or extend the incubation time
   - *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.** For large DNA fragments (>10kb), using preheated (60ºC-70ºC) elution buffer may improve the elution efficiency

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.** In case of DNA denaturation (which can be detected by gel analysis), incubate eluted DNA at 95ºC for 2 minutes, and then cool down slowly to re-anneal the DNA.
     - **iii.** In case of Low A260/280, repeat the wash step with 600µl of Wash Buffer 2, and let stand for 1 minute prior to centrifugation.