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A Geno Technology, Inc. (USA) brand name

G-CAPSULE™

For Extracting Nucleic Acids & Proteins from Agarose Gel

PRINCIPLE

G-CAPSULE™ is an electroelution tool for rapid recovery of PCR products, DNA fragments and proteins from agarose and polyacrylamide gels. G-CAPSULE™ consists of two main parts (Figure 1), G-PICK™ for picking DNA or protein band from the gel, and G-TRAP™, which traps the migrating DNA or protein during electroelution. After excising the DNA or protein band from the gel with G-PICK™, the G-CAPSULE™ assembly is then placed in a gel box and a current applied. Under the influence of an electrical field, DNA and protein molecules migrate toward the positive (+) terminal of the electrophoresis apparatus and captured by the G-TRAP™ and become bound to the membrane .

Electroelution with G-CAPSULE™ is a gentle method that eliminates the risks of damage to DNA or protein samples commonly encountered in use of glass milk, spin columns, binding columns and other popular techniques.
The G-CAPSULE™ method is simple to perform and eliminates additional steps involving washing, spinning, heating or precipitation. The recovered DNA is of highest quality for use in molecular manipulations such as ligation, restriction enzyme digestion, sequencing, amplification, random priming, and other enzymatic reactions.

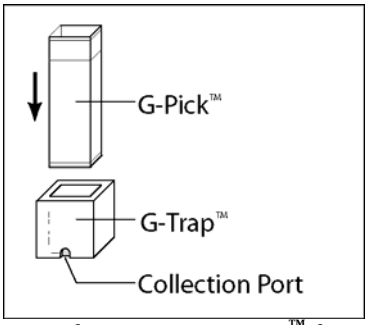


Figure 1: *G-CAPSULE™ consists of two parts: G-PICK™ for picking DNA or protein band from the gel, and G-TRAP™ for trapping migrating DNA or protein during electroelution.*

ITEM(S) SUPPLIED Cat# 786-001

G-CAPSULE™	55
Capillary Pipette Tip	55
Pin	55
Plunger	1

ADDITIONAL ITEMS NEEDED

- Horizontal electrophoresis apparatus
- TAE buffer for DNA
- Optional: G-CAPSULE™ Accessory Kit (Cat. # 786-004) Prevents G-CAPSULE™ from floating during electrophoresis (Figure 2). The G-CAPSULE™ Weight is designed to trap G-CAPSULE™ when submerged under buffer and prevents it from floating or changing direction during electroelution.

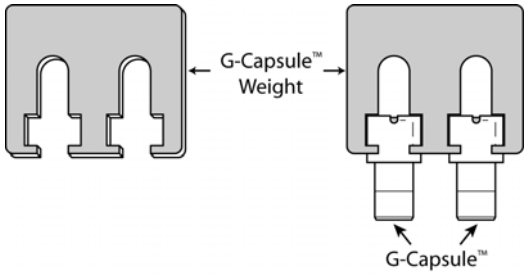


Figure 2: *G-CAPSULE™ Weight prevents 1-2 G-CAPSULE™ from floating or moving during electroelution.*

PROTOCOL SUMMARY

I. Washing: Wash G-CAPSULE™ thoroughly in elution buffer.
II. Excising and Picking up a Gel Band: Use G-PICK™ to punch out the gel band (Figure 3).
III. Assembling G-CAPSULE™: Fill G-TRAP™ with elution buffer. Assemble G-PICK™ and G-TRAP™ together. Use the plunger to push the gel piece close to the membrane. Fill G-PICK™ with elution buffer.
IV. Electroelution: Submerge G-CAPSULE™ in the gel tank and apply desired current for electroelution.
V. Recover Sample: Reverse current for 10-20 seconds. Remove the entire buffer from G-PICK™. Puncture a small hole in the Collection Port, insert the capillary tip and remove the sample collected against the membrane.

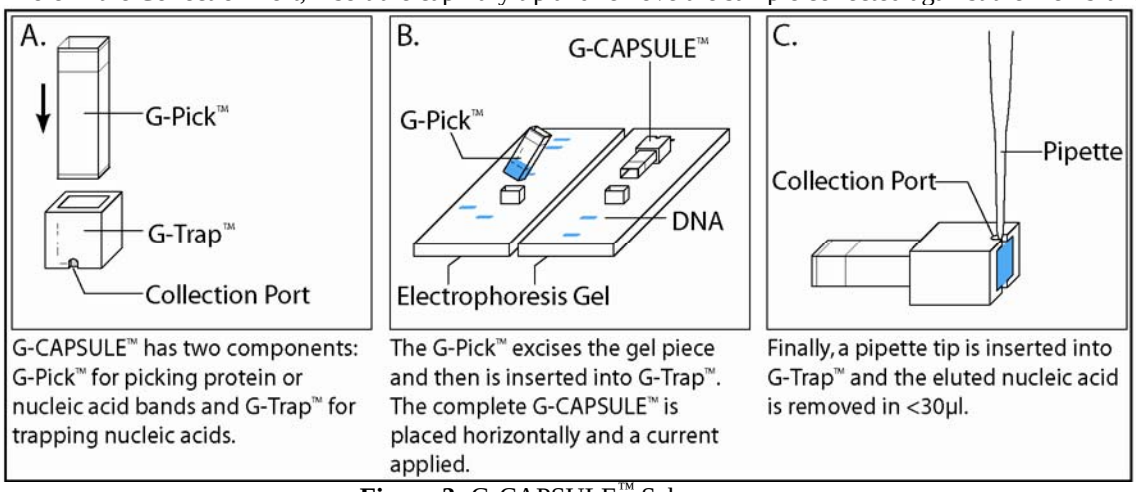


Figure 3: G-CAPSULE™ Scheme.

PROTOCOL: DNA ELECTROELUTION

Important Notes:

- *If you are using G-CAPSULE™ for the first time, we recommend performing a few trial runs on molecular weight marker bands to become familiar with the G-CAPSULE™ protocol. The critical steps are removing the free buffer from the G-PICK™, and removing the eluted sample from the collection port of the G-TRAP™.*
- *Use TAE buffer as elution buffer for DNA samples to ensure that subsequent ligation, restriction or sequencing reactions are successful. TBE buffer is known to inhibit T4 DNA Ligase and may inhibit the enzyme that catalyzes other recombinant reactions. However, TBE and any other buffer of your choice can also be used, if the enzyme inhibition is not a concern.*

I. Wash G-CAPSULE™ components:

1. Disassemble the G-CAPSULE™ unit and fill the G-TRAP™ with ~ 500µl TAE buffer. Submerge both G-PICK™ and G-TRAP™ in TAE buffer for 10-15 minutes to wash the components and to equilibrate its membrane.

2. Remove the G-PICK™ and G-TRAP™ from the buffer and wash the membrane of the G-TRAP™ 2-3 times with TAE buffer.

3. Washed G-TRAP™ can be kept in TAE buffer until use. If not used right away, store at 4°C in 0.5% sodium azide solution.

4. Immediately before use, place the G-TRAP™ on a clean surface and fill with TAE buffer or elution buffer of choice until the G-TRAP™ overflows.

II. Excising DNA band:

Note: *To visualize the DNA bands, stain the gel with ethidium bromide or other suitable fluorescent stain and observe the gel under ultraviolet light.*

1. Position the tapered, cutting edge over the DNA band to be excised.

2. Push the G-PICK™ into the soft gel until you hit the bottom hard surface of the gel tray.

3. Remove the gel band by tilting the G-PICK™ away from you and slowly pulling out of the gel.
   Note: *If there are still remnants of the DNA band left behind in the gel or you fail to pick up the DNA band properly, simply invert the G-PICK™ and use the other end to pick up the remnant of the band. Alternatively, use a sharp scalpel to trim the remnant of the DNA band and load the trimmed gel pieces into the G-PICK™.*

III. ASSEMBLING THE G-CAPSULE™

Note: *Assemble the G-CAPSULE™ immediately before you are ready for the electroelution.*

1. Ensure the G-TRAP™ is filled with elution buffer (See step I-4). If not, fill with elution buffer.

2. Position the G-PICK™, containing the DNA band, in the G-TRAP™ (see Figure 3A) by inserting the tapered, cutting edge of the G-PICK™ into the non-membrane side of the G-TRAP™. Push the G-PCIK™ until it locks in place.

3. Ensure there are no air bubbles trapped in the G-TRAP™. Remove any air bubbles by pipetting buffer into the G-CAPSULE™.

4. Using the plunger provided, push the gel piece(s) close to the membrane, so that the gel piece is uniformly in contact with the membrane.
   Note: *Do not push the plunger too hard or damage to the membrane may occur resulting in loss of sample.*

IV. ELECTROELUTION OF DNA

1. Fill the G-PICK™ with elution buffer.
   Note: *Make sure there is no air bubbles trapped in the G-PICK™ or between gel pieces.*

2. Submerge the assembled G-CAPSULE™ in buffer on top of the gel bed of the electrophoresis box such that the G-TRAP™ is facing the (+)-terminal (red color terminal).

3. Add pre-chilled electrophoresis buffer to the gel electrophoresis box to cover the G-PICK™. Remove some buffer if the G-CAPSULE™ floats.
   Optional: *G-CAPSULE–Weight (Cat. # 786-004) can be used for holding the G-CAPSULE™ once submerged.*

   Note: *Make sure the G-TRAP™ is facing the (+)-terminal, so that the DNA migrates into the G-TRAP™.*

4. Turn on the power supply to electroelute the DNA. For elution time, consult the Guide For DNA Elution Time.

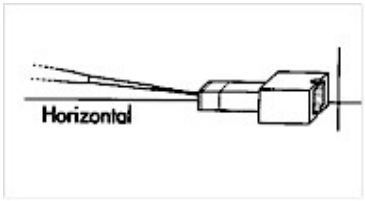
V. RECOVERING THE ELECTROELUTED DNA:

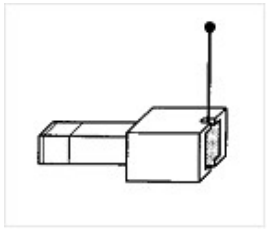
1. After electroelution is completed, adjust the voltage to ~120 V. Reverse the polarity of the current for 15-20 seconds to loosen the DNA from the membrane.

2. Turn off the power supply and remove the G-CAPSULE™ from the gel box.
   ***Note*:** *Recover the DNA immediately after turning off the power supply in order to avoid diffusion of the eluted DNA. To minimize loss of the eluted DNA, do not separate the G-PICK™ from the G-TRAP™.*

3. Hold the G-CAPSULE™ horizontally between your fingers and insert the provided pipette tip, until you reach the gel piece, then remove all the free TAE buffer from the G-PICK™ (Figure 4). Any buffer left in the G-PICK™ will be extracted with the eluted DNA and will result in a more dilute sample.
   Warning: *Be careful not to hit or disturb the gel piece in the G-CAPSULE™. If the gel band is disturbed, the DNA in the vicinity of the membrane may flow backward.*

   Note: *Removing the free TAE buffer from the G-PICK™ relieves the pressure inside the capsule so that the buffer will not leak and carry away the electroeluted DNA. Once the free buffer is removed, surface tension will keep the electroeluted molecules in the vicinity of the membrane.*





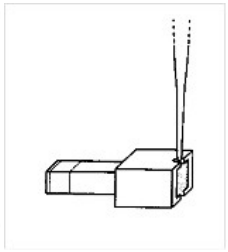


Figure 4 **Figure 5** **Figure 6**

4. Carefully place the G-CAPSULE™ on a dry surface and puncture a small hole in the membrane of the Collection Port with the supplied pin (see Figure 5).
   Warning: *Do not force the pin downward into the membrane; this will damage the membrane and spill the DNA.*

5. Pre-set a pipettor to 50-60µl, and insert the provided capillary pipette tip through the hole in the membrane and gently push the tip downward (See Figure 6). The capillary tip slides over the cutting edge (slope) of the punch. The tip pushes the wet membrane forward as it moves downward into the collection port.

6. Pipette out the DNA collected near the membrane.
   Warning: *Do not remove the G-PICK™ as this may lead to sample loss.*

7. DNA with molecular weights larger than 4000bp is efficiently eluted and recovered using G-CAPSULE™. Recovery for 2µg of DNA is generally between 80-90%. The DNA recovered from the G-CAPSULE™ is ready for most enzymatic uses. However, some grades of agarose have sulfate and other enzyme inhibitors as contaminants. If you are in any doubt, it is recommended that you precipitate the DNA by any standard method of your choice.

GUIDE FOR DNA ELUTION TIME:

Elution of DNA depends on DNA size, concentration of gel, buffer type and electrical current. It is not possible to provide a universal DNA elution time. The following table is simply a guide and will give you some indication of probable elution time in TAE buffer.

Gel (%)	DNA Size (bp)	Voltage	Elution Time (mins)
2	4,500	215 V	5
	2,500	215 V	2
	1,500	215 V	1
1	23,000	215 V	8
	9,500	215 V	6
	6,550	215 V	4
	2,500	215 V	1
0.5	23,000	215 V	7
	9,500	215 V	4
	6,500	215 V	3

NOTE: *For appropriate elution time, make test runs with the appropriate size of DNA fragments. If you are not sure of elution time, progress of DNA elution can also be monitored under UV light. Use hand held UV lamps.*

RNA ELECTROELUTION

For RNA use, the G-CAPSULE™ must be made RNase-free by treating with DEPC [diethylpyrocarbonate] or other RNase removing agents. We recommend our RNaseOUT™ (Cat. # 786-70) for removal of RNase.
Follow the protocol for DNA electroelution, except at step V.1 reverse the current at 100V for 1 min.

PROTEIN ELECTROELUTION

- *G-CAPSULE*™ can also be used for the electroelution of >4000Da proteins from polyacrylamide gels for protein sequencing, enzyme assays, raising antibodies and other protein studies.
- A horizontal electrophoresis tank is required for electroelution.
- Avoid overheating *(SEE TROUBLESHOOTING)*. If your gel box is not fitted with a cooling device, electroelution should be performed in cold-room and at a low current.
- For protein work, it is strongly recommended that G-CAPSULE™ is first soaked for 30 minutes in an appropriate buffer containing 5mM EDTA and 5% BSA solution. Wash extensively with deionized water to remove free BSA.

FOR THIN PROTEIN GELS:

Protein gels are often very thin and you might encounter difficulty in picking protein bands into the *G-PICK*™. You can overcome this problem by first picking a blank piece of agarose gel.
Protein Purification: You can purify protein from both non-denaturing polyacrylamide gel and denaturing SDS polyacrylamide gels. Use appropriate buffers for electroelution.

TROUBLESHOOTING:

Problem	Suggestion(s)
Band not completely excised	▪ Turn the G-PICK™ around and use the other open end to pick up the remnants of the band. ▪ Or after punching out a band, position the G-PICK™ in the G-TRAP™ and push the gel piece against the membrane with the plunger. Use a scalpel or any sharp instrument to trim the remaining DNA band from the gel. ▪ Cut the gel band into small pieces and load the pieces into the G-PICK™. Perform electroelution as usual. The elution time will be longer.
DNA recovery is poor	▪ The gel piece was disturbed while removing buffer from the G-PICK™. ▪ Electrical current was not reversed or reverse current was allowed to flow longer than 20-25 seconds. ▪ The membrane was damaged. ▪ Elution time was not sufficient or was excessive. ▪ DNA was degraded. ▪ The G-CAPSULE™ was vertical for a longer period, and backward flow of DNA occurred. ▪ Any manipulation or prolonged observation was performed before removing all free buffer from the G-PICK™. ▪ After electroelution, G-CAPSULE™ was placed on an absorbing or wet surface or in a puddle of fluid.
Band is wider than G-PICK™	▪ Punch out two or more gel pieces from the band, one on top of another. When punching out more than one piece from a gel band. ▪ Use the other cutting end of the G-PICK™ and punch out the remnants of the band. Depending on the thickness of the gel, you may be able to punch out 3-10 pieces of gel. ▪ Or punch out two or more gel pieces as above. Position the G-PICK™ in the G-TRAP™ and use the plunger to push the gel pieces against the membrane as described. ▪ Use a scalpel or any sharp instrument to trim the remaining DNA from the gel and load the pieces into the G-PICK™. Perform electroelution as usual. The elution time will be longer.
DNA band is crowded by other bands, which may lead to cross contamination	▪ Use a scalpel or any sharp instrument to cut out the band you intend to extract. ▪ Cut the gel band into small pieces and load the pieces into the G-PICK™. Electroelute as usual. Elution time will be longer.
Gel is too thin, leading to difficulty in picking gel bands with G-PICK™	▪ First, pick a blank piece of agarose gel into the G-PICK™ from a prepared 4-5mm thick agarose gel in appropriate buffer. ▪ Position the G-PICK™ in the G-TRAP™ and push the blank gel pieces against the membrane with the plunger. ▪ Cut the band with a sharp instrument and load into the G-PICK™.
Gel lanes are too close to each other	▪ Load samples in alternate sample wells. Alternatively, do not load samples in the wells adjacent to your most critical samples.
Want to avoid overheating	▪ Before electroelution, replace the buffer in the gel box with pre-chilled buffer. ▪ Run the electroelution in a cold room.
Elution volume is larger than 35µl	▪ The electrophoresis gel was thicker than 5-6mm. ▪ The gel piece was damaged, which increased the dead volume of elution buffer in the G-PICK™. ▪ The entire buffer was not removed from the G-PICK™. ▪ The gel piece was not pushed close to the membrane.

Buffer is leaking from the G-TRAP™	This is normal, since G-CAPSULE™ is not watertight and buffer will leak between the plastic parts. Therefore, you must be aware of the following instructions: ▪ Assemble G-CAPSULE™ just before use and quickly submerge in the tank buffer. Keep it there until you begin electrical current for electroelution. ▪ After electroelution is complete and as soon as you remove G-CAPSULE™ from the buffer, you must first remove all free buffer from G-PICK™. This will relieve pressure so fluid does not leak and carry DNA with it. Once the free buffer is removed, surface tension will keep the electroeluted DNA in the vicinity of the membrane. ▪ Remove drops of buffer around G-CAPSULE™. Use a pipette or tissue paper. Be careful not to suck inside buffer with tissue paper or pipette tip. ▪ Do not place G-CAPSULE™ on an absorbing or wet surface, or in a puddle of fluid; this could create capillary suction through the joining parts of G-CAPSULE™ filled with buffer. ▪ After electroelution, you may place G-CAPSULE™ on a dry surface, provided that you have followed the above instructions.

G-CAPSULE is protected by US Patent # 5635045

RELATED PRODUCTS

1. **Nucleic dotMETRIC™** (Cat # 786-61) *Allows DNA, RNA and oligonucleotide concentrations to be measured using as little as 1 µl of sample. Measurements take 2 minutes and are perfect for geneEXIT™ or any other use requiring a minimal waste of sample.*
2. **Gel Loading Dye** *Ready to use dyes for running agarose gel electrophoresis of DNA and RNA. The dyes for DNA are Ficoll based and are available as Glow Loading Dye and Universal Loading Dye..*
3. **Mini Horizontal Gel Electrophoresis System** (Cat# MT-108 and MT-109)**.**
4. **SpinOUT™ Columns** (Cat. # 786-170 to 786-173, 786-703 to 786-708). *The SpinOUT™ GT-600 and GT-1200 columns are versatile, spin-format columns for the desalting and buffer exchange of protein solutions ranging from 5µl through to 4ml sample volumes.*
5. **Tube-O-DIALYZER™** (Cat. # 786-610 to 786-624) *Allows dialysis of small samples without having to take the sample out of the tube thus eliminates loss (Medi & Micro size available with 1kDa, 4kDa, 8kDa, 15kDa & 50kDa MW cut off limits).*
6.

NOTE: *For other related products, visit our web site at www.GBiosciences.com or contact us.*

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