Genesee Scientific
A Life Science Company

Product Manual

GENE CHOICE
A Genesee Scientific Brand

Gel DNA Extraction Kit

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Introduction

The Gene Choice® Gel DNA Extraction Kit is an innovative system that radically simplifies the extraction and purification of nucleic acids from DNA agarose gels. The key to this system is the matrix that specifically, but reversibly, binds DNA or RNA under optimized conditions allowing proteins and other contaminants to be removed. Nucleic aids are easily eluted with deionized water or a low salt buffer.

Binding Capacity:
Each Micro DNA Column can bind ~10 µg of DNA.

Kit Contents

Product	SMP96-300	96-300	96-300B
Purifications	5	50	200
Micro DNA Columns	5	50	200
2 ml Collection Tubes	5	50	200
GC2 Binding Buffer	5 ml	30 ml	120 ml
GCW Buffer	2.5 ml	25 ml	3 x 25 ml
Elution Buffer	2 ml	30 ml	30 ml
User Manual	✓	✓	✓

Preparing Reagents

Dilute GCW Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
SMP96-300	10 ml
96-300	100 ml
96-300B	100 ml per bottle

Storage and Stability

All of the kit components are guaranteed for at least 12 months from the date of purchase when stored at room temperature.

Guidelines for Vacuum Manifold

The following is required for use with the Vacuum/Spin Protocol:

A) **Vacuum Manifold**
Other Compatible Vacuum Manifolds: Qiagen QIAvac24, Sigma AldrichVM20, Promega Vacman®, or manifold with standard Luer connector

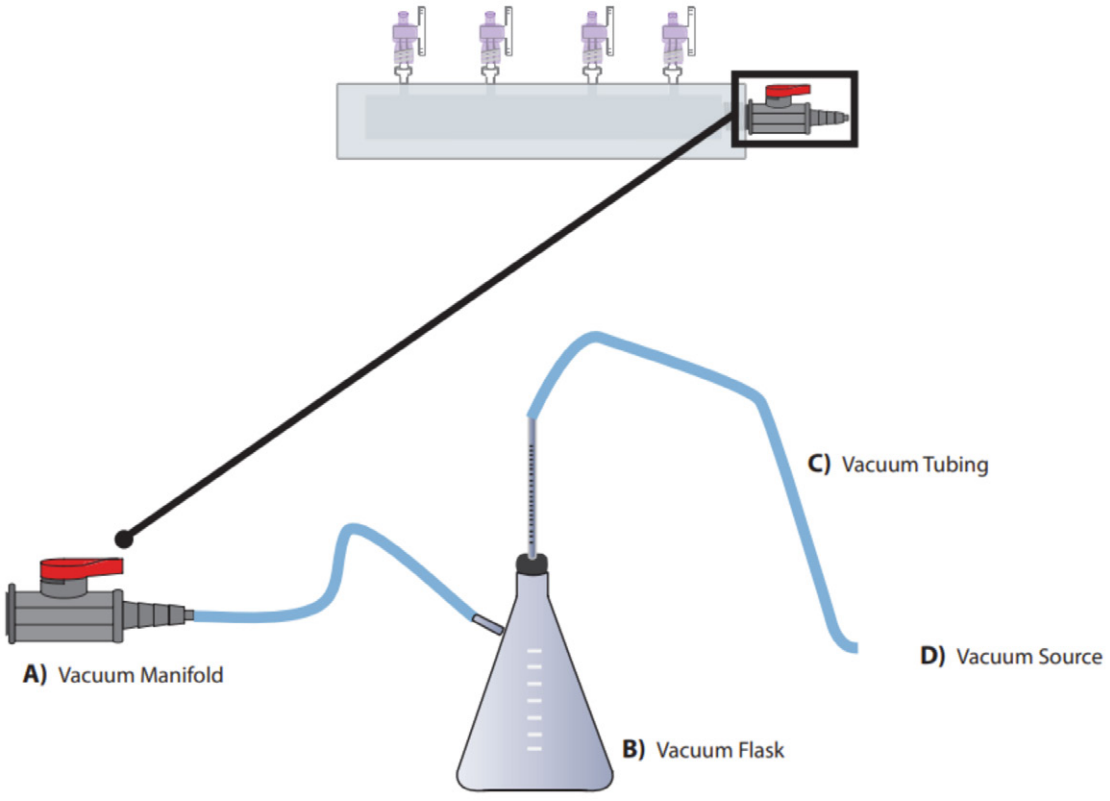
B) **Vacuum Flask**

C) **Vacuum Tubing**

D) **Vacuum Source** (review tables below for pressure settings)

Conversion from millibars:	Multiply by:
Millimeters of mercury (mmHg)	0.75
Kilopascals (kPa)	0.1
Inches of mercury (inch Hg)	0.0295
Torrs (Torr)	0.75
Atmospheres (atmos)	0.000987
Pounds per Square Inch (psi)	0.0145

Vacuum Setup:



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Centrifugation Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- 100% ethanol
- 1.5 ml microcentrifuge tubes
- Incubator capable of 55°C
- Vortexer
- Optional: 3M NaOH
- Optional: Sterile deionized water

Before Starting:

- Prepare GCW Buffer according to "Preparing Reagents" section

1. Perform agarose gel/ethidium bromide electrophoresis to fractionate DNA fragments. Any type or grade of agarose may be used. However, it is strongly recommended that fresh TAE buffer or TBE buffer be used as running buffer. Do not reuse the running buffer as its pH will increase and reduce yields

2. When adequate separation of bands has occurred, carefully excise the DNA fragment of interest using a wide, clean, sharp scalpel. Minimize the size of the gel slice by removing extra agarose

3. Determine the appropriate volume of the gel slice by weighing it in a clean 1.5 ml microcentrifuge tube. Assuming a density of 1 g/ml, the volume of gel is derived as follows: a gel slice of mass 0.3 g will have a volume of 0.3 ml

4. Add 1 volume GC2 Binding Buffer

5. Incubate at 60°C for 7 minutes or until the gel has completely melted. Vortex or shake the tube every 2-3 minutes
Important: Monitor the pH of the Gel/Binding Buffer mixture after the gel has completely dissolved. DNA yields will significantly decrease when the pH > 8.0. If the color of the mixture becomes orange or red, add 5µl 5M sodium acetate (pH 5.2) to bring the pH down. After this adjustment, the color of the Gel/Binding Buffer mixture should be light yellow.

6. Insert a Micro DNA Column in a 2 ml Collection Tube

Optional Protocol for Column Equilibration:

- Add 100 µl 3M NaOH to the Micro DNA Column
- Centrifuge at 10,000 x *g* for 30 seconds
- Add 500 µl sterile deionized water to the Micro DNA Column

7. Transfer no more than 700 µl DNA/agarose solution from **Step 5** to the Micro DNA Column
Note: Each Micro DNA Column has a total capacity of 10 µg DNA. If the expected yield is larger, divide the sample into the appropriate number of columns.

8. Centrifuge at 10,000 x *g* for 1 minute at room temperature

9. Discard the filtrate and reuse the collection tube

10. Repeat **Steps 7-9** until all the sample has been transferred to the column

11. Add 300 µl GC2 Binding Buffer

12. Centrifuge at maximum speed (≥13,000 x *g*) for 30 seconds at room temperature

13. Discard the filtrate and reuse the collection tube

14. Add 700 µl GCW Buffer
Note: GCW Buffer must be diluted with 100% ethanol prior to use.

15. Centrifuge at maximum speed for 1 minute at room temperature

16. Discard the filtrate and reuse the collection tube
Optional: Repeat **Steps 14-16** for a second GCW Buffer wash step. Perform the second wash step for any salt sensitive downstream applications.

17. Centrifuge the empty Micro DNA Column for 2 minutes at maximum speed to dry the column matrix
Note: It is important to dry the Micro DNA Column matrix before elution. Residual ethanol may interfere with downstream applications.

18. Transfer the Micro DNA Column to a clean 1.5 ml microcentrifuge tube

19. Add 10-20 µl Elution Buffer or sterile deionized water directly to the center of the column membrane
Note: The efficiency of eluting DNA from the Micro DNA Column is dependent on pH. If eluting DNA with sterile deionized water, make sure that the pH is around 8.5.

20. Let sit at room temperature for 2 minutes

21. Centrifuge at maximum speed for 1 minute
Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

22. Store DNA at -20°C

Notes

Vacuum Protocol

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- 100% ethanol
- 1.5 ml microcentrifuge tubes
- Incubator capable of 55°C
- Vacuum Manifold
- Vortexer
- **Optional:** 3M NaOH
- **Optional:** Sterile deionized water

Before Starting:

- Prepare GCW Buffer according to "Preparing Reagents" section'

1. Perform agarose gel/ethidium bromide electrophoresis to fractionate DNA fragments. Any type or grade of agarose may be used. However, it is strongly recommended that fresh TAE buffer or TBE buffer be used as running buffer. Do not reuse the running buffer as its pH will increase and reduce yields

2. When adequate separation of bands has occurred, carefully excise the DNA fragment of interest using a wide, clean, sharp scalpel. Minimize the size of the gel slice by removing extra agarose

3. Determine the appropriate volume of the gel slice by weighing it in a clean 1.5 ml microcentrifuge tube. Assuming a density of 1 g/ml, the volume of gel is derived as follows: a gel slice of mass 0.3 g will have a volume of 0.3 ml

4. Add 1 volume GC2 Binding Buffer

5. Incubate at 60°C for 7 minutes or until the gel has completely melted. Vortex or shake the tube every 2-3 minutes
Important: Monitor the pH of the Gel/Binding Buffer mixture after the gel has completely dissolved. DNA yields will significantly decrease when the pH > 8.0. If the color of the mixture becomes orange or red, add 5 µl 5M sodium acetate (pH 5.2) to bring the pH down. After this adjustment, the color of the Gel/Binding Buffer mixture should be light yellow.

6. Prepare the vacuum manifold according to manufacturer's instructions

7. Connect the Micro DNA Column to the vacuum manifold

Optional Protocol for Column Equilibration:

- Add 100 µl 3M NaOH to the Micro DNA Column
- Switch on vacuum source to draw the buffer through the column
- Turn off the vacuum
- Add 500 µl sterile deionized water to the Micro DNA Column
- Switch on vacuum source to draw the water through the column
- Turn off the vacuum

8. Add no more than 700 µl DNA/agarose solution from **Step 5** to the Micro DNA Column

9. Turn on the vacuum source to draw the sample through the column

10. Turn off the vacuum

10. Repeat **Steps 8-10** until all of the sample has been transferred to the column

12. Add 300 µl GC2 Binding Buffer

13. Turn on the vacuum source to draw the sample through the column

14. Turn off the vacuum

15. Add 700 µl GCW Buffer
Note: GCW Buffer must be diluted with 100% ethanol prior to use. Please see the "Preparing Reagents" section for instructions.

16. Turn on the vacuum source to draw the sample through the column

17. Turn off the vacuum

18. Repeat **Steps 15-17** for a second GCW Buffer wash step

19. Transfer the Micro DNA Column to a clean 1.5 ml microcentrifuge tube

20. Centrifuge the empty Micro DNA Column for 2 minutes at maximum speed to dry the column matrix
Note: It is important to dry the Micro DNA Column matrix before elution. Residual ethanol may interfere with downstream applications.

21. Transfer the Micro DNA Column to a clean 1.5 ml microcentrifuge tube

22. Add 10-20 µl Elution Buffer or sterile deionized water directly to the center of the column membrane
Note: The efficiency of eluting DNA from the Micro DNA Column is dependent on pH. If eluting DNA with sterile deionized water, make sure that the pH is around 8.5.

23. Let sit at room temperature for 2 minutes

24. Centrifuge at maximum speed for 1 minute
Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

25. Store DNA at -20°C

Notes

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, **please contact the technical support staff**.

Possible Problems and Suggestions

Problem	Cause	Solution
Low DNA Yields	• pH of the sample mixture is too high	• Add 10-20 µl sodium acetate (pH 5.2) to the sample and mix
Clogged Column in Gel Extraction	• Incompletely dissolved gel	• Increase incubation time. • Increase GC2 Binding Buffer volume
No DNA Eluted	• GCW Buffer or DNA Wash Buffer was not diluted with ethanol	• Prepare GCW Buffer or DNA Wash Buffer as instructed on **Page 4**
Optical densities do not agree with DNA yield on agarose gel	• Trace contaminants eluted from column increase A 260	• Wash column as instructed. Alternatively, rely on agarose gel/ ethidium bromide electrophoresis for quantification
DNA sample floats out of well while loading agarose gel	• Ethanol not removed completely from column following wash steps	• Centrifuge column as instructed to dry before proceeding to elution

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