Empirical bioscience

Observe the Difference

TRUST THE OUTCOME

EB Pure Agarose Gel Extraction Kit

SS-EB-AGEK-5	5 preps
EB-AGEK-50	50 preps
EB-AGEK-200	200 preps

Made in Michigan

www.Empiricalbioscience.com

REGISTERED ISO 13485

EB Pure Agarose Gel Extraction Kit Table of Contents

Introduction

Gel purification of DNA is a common technique for the isolation of specific fragments from reaction mixtures. However, most methods either fail to completely remove agarose or shear DNA which can lead to problems in downstream manipulations. The EB Pure Agarose Gel Extraction Kit uses proprietary chemistry and spin column technology to recover DNA fragments between 70 bp and 20 kb with yields exceeding 85%. The DNA band of interest is excised from the gel, dissolved in Binding Buffer (XP2), and transferred to a High-Bind DNA Mini Column. Following three rapid wash steps, DNA is eluted with the Elution Buffer and is ready for other applications. DNA is suitable for ligations, PCR, sequencing, restriction digestion, or various labeling reactions. In addition, this kit can be also used to recover DNA directly from enzymatic reactions such as PCR and enzyme digestion reactions.

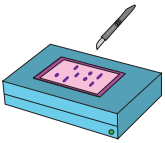
Benefits of the EB Pure Agarose Gel Extraction Kit

- **Fast -** DNA recovery from agarose gel < 10 minutes
- **Reliability -** Optimized buffers that guarantee pure DNA
- **Safety -** No organic extractions
- **Quality -** Purified DNA is suitable for most applications

Binding Capacity

Each High-Bind DNA Mini Columns can bind ~25 µg DNA.

Spin Protocol



Excise DNA Band from Gel

Incubate and Melt Gel with Binding Buffer

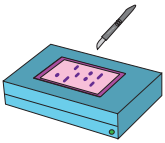
Transfer Lysate & Bind

Wash 3x

Dry

Elute

Vacuum/Spin Protocol



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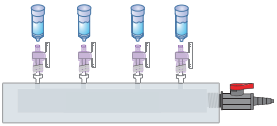


Wash 3x

Dry

Elute

Kit Contents and Storage

Product	SS-EB-AGEK-5	EB-AGEK-50	EB-AGEK-200
Purifications	5	50	200
High-Bind DNA Mini Columns	5	50	200
Collection Tubes (2 mL)	5	50	200
Binding Buffer (XP2)	5 mL	40 mL	150 mL
SPW Wash Buffer	5 mL	25 mL	3 x 25 mL
Elution Buffer	1.5 mL	10 mL	20 mL

Storage and Stability

All EB Pure Agarose Gel Extraction Kit components are guaranteed for at least 12 months from the date of purchase when stored at room temperature. Please ensure that the bottle of Binding Buffer (XP2) is tightly capped when not in use. If any precipitates form in the buffers, warm at 37°C to dissolve.

Preparing Reagents

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

Kit	100% Ethanol to be Added
SS-EB-AGEK-5	20 mL
EB-AGEK-50	100 mL
EB-AGEK-200	100 mL per bottle

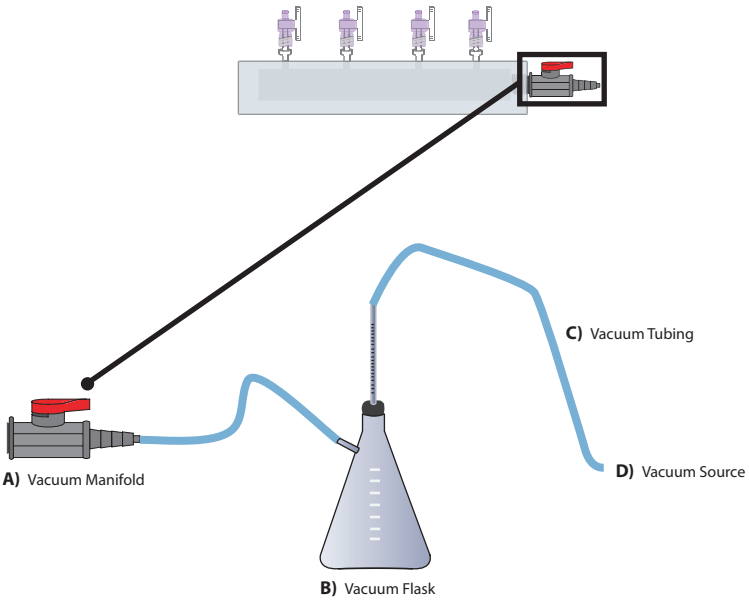
Guideline for Vacuum Manifold

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

A) Vacuum Manifold
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 (inHg)	0.0295
Torrs (Torr)	0.75
atmospheres (atm)	0.000987
pounds per square inch (psi)	0.0145

Illustrated Vacuum Setup:



EB Pure Agarose Gel Extraction Kit - Spin Protocol

Materials and Equipment to be Supplied by User:

- Heat block or water bath capable of 60°C
- Microcentrifuge capable of at least 13,000 x *g*
- Vortexer
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- Optional: 5M Sodium Acetate, pH 5.2
- Optional: Sterile deionized water

Before starting:

- Prepare SPW Wash Buffer according to the "Preparing Reagents" section on Page 5
- Set heating block or water bath to 60°C

Note: The yellow color of the Binding Buffer (XP2) signifies a pH of ≤ 7.5.

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 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 Binding Buffer (XP2).

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 (XP2) 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 (XP2) mixture should be light yellow.

6. Insert a High-Bind DNA Mini Column in a Collection Tube (2 mL).

7. Add no more than 700 µL DNA/agarose solution from Step 5 to the High-Bind DNA Mini Column.

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

9. Discard the filtrate and reuse collection tube.

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

11. Add 300 µL Binding Buffer (XP2).

12. Centrifuge at maximum speed (≥13,000 x *g*) for 1 minute at room temperature.

13. Discard the filtrate and reuse collection tube.

14. Add 700 µL SPW Wash Buffer.

    Note: SPW Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

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

16. Discard the filtrate and reuse collection tube.

Optional: Repeat Steps 14-16 for a second SPW Wash Buffer wash step. Perform the second wash step for any salt sensitive downstream applications.

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

18. Transfer the High-Bind DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

19. Add 30-50 µL Elution Buffer or deionized water directly to the center of the column membrane.
    Note: The efficiency of eluting DNA from the High-Bind DNA Mini Column is dependent on pH. If eluting DNA with 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.

EB Pure Agarose Gel Extraction Kit - Vacuum Protocol

Materials and Equipment to be Supplied by User:

- Vacuum Manifold (See Page 6)
- Heat block or water bath capable of 60°C
- Microcentrifuge capable of at least 13,000 x *g*
- Vortexer
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol
- Optional: 5M Sodium Acetate, pH 5.2
- Optional: Sterile deionized water

Before starting:

- Prepare the Vacuum Manifold (See Page 6)
- Prepare SPW Wash Buffer according to the "Preparing Reagents" section on Page 5
- Set heating block or water bath to 60°C

Note: The yellow color of the Binding Buffer (XP2) signifies a pH of ≤ 7.5.

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 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 Binding Buffer (XP2).

5. Incubate at 60°C for 7 minutes or until the gel has completely melted. Vortex or shake the tube every 2-3 minutes.

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

7. Connect the High-Bind DNA Mini Column to the vacuum manifold.

8. Add no more than 700 µL DNA/agarose solution from Step 5 to the High-Bind DNA Mini Column.

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

10. Turn off the vacuum.

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

12. Add 300 µL Binding Buffer (XP2).

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

14. Turn off the vacuum.

15. Add 700 µL SPW Wash Buffer.

    Note: SPW Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

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

17. Turn off the vacuum.

Optional: Repeat Steps 15-17 for a second SPW Wash Buffer wash step. Perform the second wash step for any salt sensitive downstream applications.

18. Transfer the High-Bind DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

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

20. Transfer the High-Bind DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

21. Add 30-50 µL Elution Buffer or deionized water directly to the center of the column membrane.
    Note: The efficiency of eluting DNA from the High-Bind DNA Mini Column is dependent on pH. If eluting DNA with deionized water, make sure that the pH is around 8.5.

22. Let sit at room temperature for 2 minutes.

23. 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.

24. Store DNA at -20°C.

EB Pure Agarose Gel Extraction Kit Protocol - Purification of DNA from Enzymatic Reactions

The following protocol is designed for DNA recovery from enzymatic reactions such as PCR and probe labeling reactions.

Materials and Equipment to be Supplied by User:

- Microcentrifuge capable of at least 13,000 x *g*
- Vortexer
- Nuclease-free 1.5 mL microcentrifuge tubes
- 100% ethanol

Before starting:

- Prepare SPW Wash Buffer according to the "Preparing Reagents" section on Page 5

Note: The yellow color of the Binding Buffer (XP2) signifies a pH of ≤ 7.5.

1. Determine the volume of the enzymatic reaction.
2. Transfer the sample into a clean 1.5 mL microcentrifuge tube.
3. Add 1 volume Binding Buffer (XP2).
4. Vortex or invert the sample to mix thoroughly.
5. Briefly centrifuge the tube to collect any drops from the inside of the lid.
6. Insert a High-Bind DNA Mini Column into a Collection Tube (2 mL).
7. Transfer the sample to the High-Bind DNA Mini Column.
8. Centrifuge at 10,000 x *g* for 1 minute at room temperature.

9. Discard the filtrate and reuse the collection tube.

10. Add 700 µL SPW Wash Buffer.
    Note: SPW Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 5 for instructions.

11. Centrifuge at maximum speed (≥13,000 x *g*) for 1 minute at room temperature.

12. Discard the filtrate and reuse collection tube.

13. Repeat Steps 10-12 for a second SPW Wash Buffer wash step.

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

15. Transfer the High-Bind DNA Mini Column to a clean 1.5 mL microcentrifuge tube.

16. Add 30-50 µL Elution Buffer or deionized water directly to the center of the column membrane.
    Note: The efficiency of eluting DNA from the High-Bind DNA Mini Column is dependent on pH. If eluting DNA with deionized water, make sure that the pH is around 8.5.

17. Let sit at room temperature for 2 minutes.

18. 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.

19. Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise.

Possible Problems and Suggestions

Low DNA Yields	
Too little Binding Buffer (XP2) added to gel	Volume of agarose gel slice determined incorrectly. Add enough Binding Buffer (XP2) as instructed.
Agarose gel does not completely dissolve	Make sure water bath is set to 60°C and allow gel to completely melt. Add more XP2 Buffer if necessary.
Inappropriate elution buffer	Use Elution Buffer or check the pH of the water used to elute DNA.
TAE/TBE running buffer is not fresh	With overuse, TAE buffer loses its buffering capacity and its pH increases. This raises the pH of the agarose/Binding Buffer (XP2) solution which interferes with DNA binding to the column matrix. Adjust pH by adding 5 µL 5M sodium acetate, pH 5.2, to the gel slice. Use freshly prepared TAE buffer for gel purification in order to prevent the contamination of isolated DNA and improve yields.
Column clogged	
Agarose gel not completely dissolved in Binding Buffer (XP2)	Make sure water bath is set to 55-60°C and allow gel to completely melt. For large agarose slices (>0.3 mL), it is recommended that the gel be diced into smaller fragments to aid melting.
No DNA eluted	
SPW Wash Buffer not diluted with 100% ethanol	Prepare SPW Wash Buffer as instructed on page 5 or as indicated on bottle.
Incorrect amount of XP2 Buffer added	Measure the gel accurately and use 0.1 mL XP2 Buffer per 0.1 g gel.
Optical densities do not agree with DNA yield on agarose gel	
Trace contaminants eluted from column increase A_{260}	Make sure to wash column as instructed in Step 14 of the spin protocol, and Step 15 of the vacuum protocol. Alternatively, rely on agarose gel/ethidium bromide electrophoresis for quantization.
DNA sample floats out of well while loading agarose gel.	
Ethanol not completely removed from column	Centrifuge as instructed in Step 17 of the spin protocol and Step 19 of the vacuum protocol.