GenElute™ HP Five–Minute Plasmid Miniprep Kit

SIGMA Life Science

User Guide

Catalog Nos.

PFM10

PFM50

PFM250

Ordering Information

Catalog No.	Product Description	Pkg Size
PFM10	GenElute Five-Minute Plasmid Miniprep Kit	10 preps
PFM50	GenElute Five-Minute Plasmid Miniprep Kit	50 preps
PFM250	GenElute Five-Minute Plasmid Miniprep Kit	250 preps

Related Products

Catalog No.	Product Description	Pkg Size
PLN10	GenElute Plasmid Miniprep Kit	10 preps
PLN70	GenElute Plasmid Miniprep Kit	70 preps
PLN350	GenElute Plasmid Miniprep Kit	350 preps
NA0200S	GenElute HP Plasmid Midiprep Kit	4 preps
NA0200	GenElute HP Plasmid Midiprep Kit	25 preps
NA0300S	GenElute HP Plasmid Maxiprep Kit	4 preps
NA0300	GenElute HP Plasmid Maxiprep Kit	10 preps
NA0310	GenElute HP Plasmid Maxiprep Kit	25 preps
NA0400S	GenElute HP Endotoxin-Free Plasmid Maxiprep Kit	4 preps
NA0400	GenElute HP Endotoxin-Free Plasmid Maxiprep Kit	10 preps
NA0410	GenElute HP Endotoxin-Free Plasmid Maxiprep Kit	25 preps
NA0500	GenElute HP Plasmid Megaprep Kit	5 preps
NA0600	GenElute HP Endotoxin-Free Plasmid Megaprep Kit	5 preps

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GenElute™ Five–Minute Plasmid Miniprep Kit

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Product Description

The GenElute Five-Minute Plasmid Miniprep Kit offers a rapid and simplified method for isolating plasmid DNA from recombinant *E. coli* cultures. The kit employs a novel procedure that eliminates many conventional steps in plasmid preparation, such as cell harvesting, resuspension, alkaline lysis, and lysate clearing. With this streamlined procedure, plasmid DNA can be recovered directly from *E. coli* culture in 5 minutes.

Recombinant *E. coli* overnight culture in LB (Luria-Bertani) broth is treated briefly (1–2 minutes) with a lysis reagent to effect rapid cell lysis and RNA degradation, without prior removal of culture medium. A binding solution is then mixed with the lysate, and DNA is captured on a silica-based binding column. Impurities are removed by a wash solution and bound plasmid DNA is then eluted in Tris buffer or water, ready for immediate use in various downstream applications.

The GenElute Five-Minute Plasmid Miniprep Kit is intended for rapid preparation of plasmid DNA for use in capillary DNA sequencing, clone screening, restriction digestion, and PCR. Typical yields of high copy plasmids are 2–6 µg from 400 µL of overnight culture in LB broth. For DNA sequencing, it is recommended to quantify the concentration of plasmid DNA by agarose gel electrophoresis or by PicoGreen® assay, with 1 µl of eluate. For low copy plasmids, the kit can be used to prepare plasmid DNA for use in restriction digestion and PCR.

Components	Catalog No.	10 Prep	50 Prep	250 Prep
Lysis Reconstitution Solution	**L4292**	0.9 mL	3.5 mL	15 mL
Lysis Reagent	**L4167**	15 mg	50 mg	250 mg
Binding Solution	**B0310**	6 mL	30 mL	135 mL
Column Preparation Solution	**C2112**	7 mL	35 mL	150 mL
Wash Solution Concentrate	**W3514**	3 mL	15 mL	70 mL
Elution Solution (10 mM Tris, pH 8.5)	**E7777**	1.5 mL	8 mL	45 mL
GenElute Miniprep Binding Column	**G4669**	10 each	50 each	250 each
Collection Tubes, 2.0 mL	**T5449**	3 × 10 each	3 × 50 each	3 × 250 each

Reagents and Equipment Required But Not Provided

- Ethanol (95–100%), Catalog Nos. **E7148**, **E7023**, or **459836**
- LB medium supplemented with appropriate antibiotic
- Microcentrifuge capable of achieving 12,000 × *g* or higher
- Vacuum manifold with Luer fittings (vacuum format only)
- Vacuum source capable of 500 mbar (vacuum format only)

Precautions and Disclaimer

The GenElute Five-Minute Plasmid Miniprep Kit is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

Storage and Stability

Store the kit at room temperature. Store the reconstituted Lysis Reagent at 2–8 °C for short-term (<3 months) or at –20 °C for long-term (>3 months) storage.

Preparation Instructions

1. Grow Bacterial Cultures

The GenElute Five-Minute Plasmid Miniprep Kit is optimized for bacterial cultures grown in LB broth. Grow cultures in LB with appropriate antibiotic to an OD_{600} of 1.5–3.0 (16–19 hr). Do not overgrow the culture. For optimal results, use the cultures before they exceed an OD_{600} of 3.0. Do not use rich media, such as TB, for growing culture, as cultures in rich media can produce substantially higher cell densities, which may overload the purification system and clog the binding column.

For best results, process the cultures as soon as possible after removal from shaker. If cultures have been left on bench or stored at 2–8 °C, vortex the cultures thoroughly before proceeding to plasmid preparation.

Less than the standard 400 µl of culture per preparation can be used with proportionally reduced amount of reconstituted Lysis Reagent (1/10th of the culture volume) and Binding Solution (volume equal to the culture volume). However, culture volumes greater than 400 µl are not recommended with the vacuum format because the binding column may clog.

2. Prepare Lysis Reagent

Mix the Lysis Reconstitution Solution briefly and chill it on ice for 5–10 minutes or in a refrigerator for at least 10 minutes. Add an appropriate amount of the precooled Lysis Reconstitution Solution to the Lysis Reagent vial according to the table below, and mix thoroughly until all powder dissolves. Store the reconstituted Lysis Reagent at 2–8 °C for short-term (<3 months) or at –20 °C for long-term (>3 months) storage.

Kit Size	Amount of Lysis Reconstitution Solution to Add
10 Preps	0.75 mL
50 Preps	3 mL
250 Preps	14 mL

3. Prepare Wash Solution

Prior to first time use, add an appropriate amount of 95–100% ethanol to bottle of Wash Solution Concentrate according to the table below.

Kit Size	Amount of Ethanol to Add
10 Preps	12 mL
50 Preps	60 mL
250 Preps	280 mL

Store the diluted Wash Solution tightly capped to prevent the evaporation of ethanol.

Procedure

***Note**: All spins at maximum speed (≥12,000 × g) and at room temperature; the suggested time in each step is only a minimum and can be extended for the sake of convenience.*

I. Spin Format

1. Lyse culture

Add **40 µL of reconstituted and prechilled Lysis Reagent (see Preparation Instructions)** to 400 µL of overnight culture in a 2-mL Collection Tube (provided). Mix briefly (3–5 seconds) by rapid inversion, vortexing, or pipetting up and down. Incubate at room temperature for 2 minutes. Culture will typically become clear after 2 minutes.

Note: The lysis incubation period may be reduced to 1 minute if maximum recovery is not required for the intended application. Dense cultures (OD_{600} >3.0) and some bacterial strains (such as XL1-Blue) may require longer incubation time for optimal plasmid recovery. In such cases, continue incubation until mixture clears. Longer incubation time does not have adverse effects on plasmid DNA quality.

2. Prepare Binding Column

Insert a GenElute Miniprep Binding Column into a 2-mL Collection Tube (provided). Add **500 µL of Column Preparation Solution** to each column and spin for 10 seconds. Decant the flow-through and insert the column back into the Collection Tube for subsequent use.

Note: This step can be carried out during the lysis incubation; however, it may be more convenient to prepare Binding Columns in batch earlier in the day prior to plasmid preparation if a large number of columns are needed. The pre-washed Binding Column will stay fresh for at least one day. This simple column preparation step ensures more uniform and higher plasmid yields.

3. Bind DNA

Add **400 µL of Binding Solution** to the lysate, cap the tube, and mix thoroughly by inverting at least 15 times. **Do not vortex.** Pour the mixture or pipette approximately 780 µL of the mixture to a pre-washed Binding Column seated in a 2-mL Collection Tube and spin for 20 seconds. Decant the flow-through.

Note: Inadequate mixing of the lysate mixture after adding Binding Solution might result in lower recovery. Plasmid DNA is stable in Binding Solution. When pouring the mixture into the column, there is no need to transfer every drop. Each column can only hold approximately 780 µL with the cap closed.

4. Wash column

Important Reminder: *Prior to first time use of the kit, be sure to add ethanol to the Wash Solution Concentrate (see Preparation Instructions).*

Add 700 µL of diluted Wash Solution to each column and spin for 20 seconds. Decant the flow-through. Add another **200 µL of diluted Wash Solution** to each column and spin for 30 seconds to wash and dry the filter. Carefully remove the column from centrifuge after the drying step to avoid splashing the flow-through liquid onto the dried column. If the flow-through liquid does contact the dried column, re-centrifuge the column for 20 seconds before proceeding to the elution step.

5. Elute DNA

Transfer the Binding Column to a clean 2-mL Collection Tube (provided). Add **40 µL of Elution Solution** (or water if desired) directly to the surface of the filter and spin for 30 seconds to elute. Plasmid DNA is now present in the eluate and ready for immediate use or storage at –20 °C.

Note: If a more concentrated plasmid DNA preparation is required, reduce the volume of Elution Solution to 30 µL. However, this may result in a reduction in the total plasmid yield.

II. Vacuum Format

1. Lyse culture

Add **40 µL of reconstituted and prechilled Lysis Reagent (see Preparation Instructions)** to 400 µL of overnight culture in a 2-mL Collection Tube (provided). Mix briefly (3–5 seconds) by rapid inversion, vortexing, or pipetting up and down. Incubate at room temperature for 2 minutes. Culture will typically become clear after 2 minutes.

Note: The lysis incubation period may be reduced to 1 minute if maximum recovery is not required for the intended application. Dense cultures (OD_{600} >3.0) and some bacterial strains (such as XL1-Blue) may require longer incubation time for optimal plasmid recovery. In such cases, continue incubation until mixture clears. Longer incubation time does not have adverse effects on plasmid DNA quality.

2. Prepare Binding Column

Place GenElute Miniprep Binding Columns onto a vacuum manifold and add **500 µL of Column Preparation Solution** to each column. Turn on the vacuum to draw the liquid through. Turn off the vacuum after the liquid has flowed through.

Note: This step can be carried out during the lysis incubation; however, it may be more convenient, to prepare Binding Columns in batch earlier in the day prior to plasmid preparation if a large number of columns are needed. The pre-washed Binding Column will stay fresh for at least one day. This simple column preparation step ensures more uniform and higher plasmid yields.

3. Bind DNA and wash column

Important Reminder: *Prior to first time use of the kit, be sure to add ethanol to the Wash Solution Concentrate (see Preparation Instructions).*

Add 400 µL of Binding Solution to the lysate, cap the tube, and mix thoroughly by inverting at least 15 times. **Do not vortex**. Pipette the mixture up and down once and then pipette it into a prewashed Binding Column seated on a vacuum manifold. Turn on the vacuum. After the lysate has passed through the column, add **1 mL of diluted Wash Solution** to each column. Turn off the vacuum after the wash fluid has flowed through.

Note: Inadequate mixing of the lysate mixture after adding Binding Solution might result in lower recovery. Plasmid DNA is stable in Binding Solution. Pipetting the lysate mixture up and down once prior to transferring helps to break up large aggregates, which might otherwise cause clogging, particularly when the culture is dense.

4.	**Spin-dry column**	Remove the Binding Column from the vacuum manifold and insert it into a 2-mL Collection Tube (provided). Spin for 30 seconds to dry the filter. Carefully remove the column from the centrifuge after the drying step to avoid splashing the flow-through liquid onto the dried column. If the flow-through liquid does contact the dried column, re-centrifuge the column for 20 seconds before proceeding to the elution step.
5.	**Elute DNA**	**Elute DNA** Transfer the Binding Column to a clean 2-mL Collection Tube (provided). Add **40 µL of Elution Solution** (or water if desired) directly to the surface of the filter and spin for 30 seconds to elute. Plasmid DNA is now present in the eluate and ready for immediate use or storage at –20 °C. **Note**: If a more concentrated plasmid DNA preparation is required, reduce the volume of Elution Solution to 30 µL. However, this may result in a reduction in the total plasmid yield.

Results

For DNA sequencing, it is recommended to quantify the concentration of plasmid DNA by agarose gel electrophoresis or by PicoGreen assay, with 1 µL of eluate. Quantitation by UV absorbance is not recommended as it will result in 2 to 3–fold overestimation of plasmid DNA concentration in the eluate.

Related Products	Catalog No.	Related Products	Catalog No.
Water, Molecular Biology Reagent	**W4502**	Gel Loading Solution	**G2526**
Precast Agarose Gels, 1.0%, 8 well	**P5472**	DirectLoad™ Wide Range DNA Marker	**D7058**
TAE Buffer (10X)	**T9650**	Ethidium bromide, aqueous, 10 mg/mL	**E1510**
TBE Buffer (10X)	**T4415**	Capillary Electrophoresis Running Buffer (10X)	**B4930**
SeqSaver™ Sequencing Premix Dilution Buffer	**S3938**		

References

1. Vogelstein, B. and Gillespie, D., Preparative and analytical purification of DNA from agarose. Proc. Natl. Acad. Sci. USA, **76**, 615-619 (1979).

Troubleshooting Guide

Poor or low plasmid DNA recovery	**Cause** — Wash Solution is too concentrated. **Solution** — Wash Solution Concentrate must be diluted with the specified volume of ethanol prior to first-time use of the kit. Store the diluted Wash Solution tightly capped to prevent evaporation of ethanol.
	Cause — Culture is too old. **Solution** — Overnight cultures that are initiated from old agar plates or from cultures that have undergone several passages in the laboratory may give significant decreases in plasmid yield. Streak out a new culture from a frozen glycerol stock and inoculate an overnight culture with a single colony from the flesh plate.
	Cause — Plasmid replication is poor. **Solution** — Confirm that cells were grown in LB broth under optimal conditions.
	Cause — Antibiotic activity is insufficient. **Solution** — Use a fresh antibiotic solution for growth of overnight cultures. Most antibiotic solutions are light-sensitive and degrade during long-term storage at 2–8 °C.
	Cause — Cells not completely lysed. **Solution** — Some bacterial strains (such as XL1-Blue) may require longer than 2 minutes to lyse. Incubate longer than 2 minutes until the culture clears.
	Cause — Inadequate mixing after adding Binding Solution. **Solution** — Mix the lysate mixture thoroughly after adding Binding Solution by inverting at least 15 times.
	Cause — Binding Column not prepared with Column Preparation Solution. **Solution** — Wash binding columns with 500 µL of Column Preparation Solution before binding DNA. This simple column preparation step ensures more uniform and higher plasmid yields.

Binding column is clogged during vacuum filtration	**Cause** — Cell density is too high. **Solution** — Remove the clogged column from vacuum manifold, insert it into a 2-mL Collection Tube, and use centrifugation to complete the procedure. Use cultures before they reach OD_{600} >3.0. Do not use rich media, such as TB, for growing cultures.
	Cause — Lysate and Binding Solution are not mixed properly before transferring to the Binding Column. **Solution** — Pipette the lysate mixture up and down once to break up large aggregate before transferring it into the column.
Poor performance in downstream enzymatic applications	**Cause** — Eluate is contaminated with the flow-through from diluted Wash Solution. **Solution** — If the flow-through contacts the dried Binding Column, re-centrifuge the column for 20 seconds before proceeding to the elution step.
	Cause — Not enough or too much plasmid DNA template is used in sequencing reaction. **Solution** — Quantitate the plasmid DNA concentration by agarose gel electrophoresis or by PicoGreen assay, with 1 µL of eluate. Do not use UV absorbance for quantitation.

Notes

Notes

Experienced User Protocol: Vacuum Format

All spins at maximum speed and at room temperature.

1 Lyse Culture

- ❒ Add 40 µL of reconstituted Lysis Reagent to 400 µL of overnight culture in 2-mL Collection Tube. Mix briefly by rapid inversion and incubate for 2 minutes.

  Prior to first-time use of the kit, be sure to add Lysis Reconstitution Solution to the Lysis Reagent vial.

2 Prepare Binding Column

- ❒ Add 500 µL of Column Preparation Solution to a Binding Column seated in a vacuum manifold and turn on the vacuum. Turn off the vacuum after the liquid has passed through.

3 Bind DNA and Wash Column

- ❒ Add 400 µL of Binding Solution and mix by inversion at least 15 times.
- ❒ Pipette the mixture up and down once and then pipette the mixture to a pre-washed Binding Column seated in a vacuum manifold and turn on the vacuum.
- ❒ After the liquid has flowed through, add 1 mL of diluted Wash Solution to each column. Turn off the vacuum after the Wash Solution has passed through.

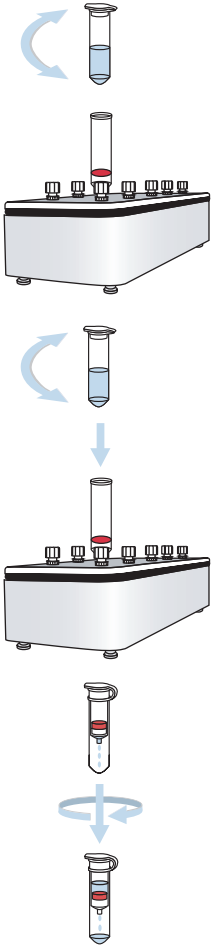
  Prior to first-time use of the kit, be sure to add ethanol to the Wash Solution Concentrate.

4 Spin-Dry Binding Column

- ❒ Transfer column to a new 2-mL Collection Tube and spin 30 seconds to dry column.

5 Elute Plasmid DNA

- ❒ Carefully transfer column to a new 2-mL Collection Tube.
- ❒ Add 40 µL of Elution Solution and spin 30 seconds.



Experienced User Protocol: Spin Format

All spins at maximum speed and at room temperature.

1 Lyse Culture

- ❒ Add 40 µL of reconstituted Lysis Reagent to 400 µL of overnight culture in 2-mL Collection Tube. Mix briefly by rapid inversion and incubate for 2 minutes.

  Prior to first-time use of the kit, be sure to add Lysis Reconstitution Solution to the Lysis Reagent vial.

2 Prepare Binding Column

- ❒ Add 500 µL of Column Preparation Solution to a Binding Column seated in a 2-mL Collection Tube. Spin 10 seconds and discard flow-through.

3 Bind DNA

- ❒ Add 400 µL of Binding Solution and mix by inversion at least 15 times.
- ❒ Pour or pipette (~780 µL) the mixture to a pre-washed binding column and spin 20 seconds. Discard flow-through.

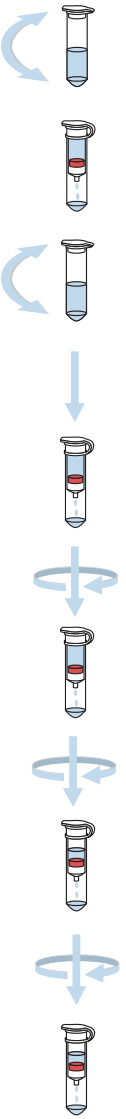
4 Wash to Remove Impurities

- ❒ Add 700 µL of diluted Wash Solution to column and spin 20 seconds. Discard flow-through.
- ❒ Add 200 µL of diluted Wash Solution to column and spin 30 seconds.

  Prior to first-time use of the kit, be sure to add ethanol to the Wash Solution Concentrate.

5 Elute Plasmid DNA

- ❒ Carefully transfer column to a new 2-mL Collection Tube.
- ❒ Add 40 µL of Elution Solution and spin 30 seconds.



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