Analyzing DNA yield and quality

Measure DNA concentration using UV absorbance at 260 nm or Qubit™ DNA Assay Kits (Cat Nos. Q32853 and Q 32854). Qubit™ DNA Assay Kits provide a rapid, sensitive, and specific fluorescent method for measuring dsDNA concentration. The kits provide a state of the art quantitation reagent, DNA standards for standard curve, and pre-made buffer.

Typically, DNA purified using the Purelink™ Quick Plasmid Miniprep Kit has an A_{260}/A_{280} of >1.80 when samples are diluted in Tris-HCl (pH 7.5), indicating that the DNA is reasonably clean of proteins that could interfere with downstream applications. Confirm absence of contaminating genomic DNA and RNA using agarose gel electrophoresis.

Troubleshooting

Problem	Solution
Low plasmid DNA yield	Use high copy number plasmids and appropriate growth conditions. For low copy number plasmids, increase the amount of culture and process as separate samples, if needed.
	Carefully remove all media before resuspending the bacterial cell pellet. Ensure complete suspension of the pellet.
	If the lysate is viscous, reduce the amount of cells used.
Denatured plasmid DNA	Do not incubate the lysate at room temperature for more than 5 minutes before adding Precipitation Buffer (N4). Denatured DNA appears as a band just above the supercoiled plasmid DNA. Restriction enzymes will not digest denatured DNA.
Contaminating Genomic DNA	Gently invert the tubes to mix the solution after adding Buffer L7. Do not exceed 5 minutes incubation before adding Precipitation Buffer (N4).
Contaminating RNA	Make sure that RNase A is added to Resuspension Buffer (R3). Store Buffer R3 with RNase A at 4°C for no longer than 6 months.
Enzymatic reactions are inhibited	Centrifuge the column to completely dry the column and remove any residual Wash Buffer (W9). Discard the flowthrough.
Slow column flow (using vacuum)	Ensure that the vacuum manifold is attached to a vacuum source and that unused luer extensions are closed.

For support visit **thermofisher.com/support**

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PureLink™ Quick Plasmid Miniprep Kits

invitrogen

QUICK REFERENCE

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Doc. Part No. 25-0789

Package Contents

Catalog Numbers	Amount:
K210010	50 reactions
K210011	250 reactions

Storage Conditions

- Store all components at room temperature. If not using the spin columns within 6 months after purchase, store the spin columns at 4°C.

Required Materials

- Compatible vacuum manifold with vacuum source capable of –600 to –800 mbar (vacuum protocol only)
- 100% isopropanol
- 100% ethanol
- Microcentrifuge capable of >12,000 × *g* at room temperature.

Timing

Bacterial culture: overnight
Purification: 30-45 minutes

Selection Guide

Go online to view related products:

Product Description

The PureLink™ Quick Plasmid Miniprep Kits enable isolation of high quality plasmid DNA (up to 40 µg) from *E. coli* cells in 30-45 minutes. Purified plasmid DNA is suitable for all routine downstream applications including bacterial cell transformation, mammalian cell transfection, DNA sequencing, restriction enzyme digestion, cloning, and PCR. The PureLink® Quick Plasmid Miniprep Kit can be used with a centrifuge or a vacuum manifold.

Important Guidelines

- Add RNase A to Resuspension Buffer (R3) according to the instructions on the label. Mix well. Mark the bottle label after adding RNase A. Store Buffer R3 with RNase A at 4°C for up to 6 months. It is recommended to store columns at 4°C for longer periods.
- Warm Lysis Buffer (L7) briefly at 37°C to redissolve any particulate matter.
- Add 96–100% ethanol to Wash Buffer (W9) and Wash Buffer (W10) according to instructions on each label. Mix well. Store wash buffers with ethanol at room temperature.
- If using a vacuum manifold, set up and attach the manifold to a vacuum source.
- Grow transformed *E. coli* in 1–5 mL LB medium overnight.

Online Resources

Visit our product page for additional information and protocols. For support, visit **thermofisher.com/support**.

For Research Use Only. Not for use in diagnostic procedures.

Miniprep plasmid isolation protocol (centrifuge)

Follow this procedure to purify plasmid DNA using a centrifuge. Use a microcentrifuge capable of centrifuging at >12,000 × g. For processing a large number of samples simultaneously, see the "Miniprep plasmid isolation protocol (vacuum)".

- Perform all centrifugation steps at room temperature using a microcentrifuge.
- Optional: Preheat an aliquot of TE Buffer (TE) to 65–70°C for eluting DNA. Heating is optional for eluting 1–30 kb plasmid DNA but is recommended for eluting DNA >30 kb.
- **Ensure the bag containing the PureLink™ Quick Spin Columns is closed tightly after each use.**
- **Caution: Buffers contain hazardous reagents. Use caution when handling buffers.**

Steps		Procedure Details
1	**Harvest**	Centrifuge 1–5 mL of the overnight LB-culture. (Use 1–2 ×10^9 *E. coli* cells for each sample.) Remove all medium.
2	**Resuspend**	Add 250 µL Resuspension Buffer (R3) with RNase A to the cell pellet and resuspend the pellet until it is homogeneous.
3	**Lyse**	Add 250 µL Lysis Buffer (L7). Mix gently by inverting the capped tube until the mixture is homogeneous. Do not vortex. Incubate the tube at room temperature for 5 minutes.
4	**Precipitate**	Add 350 µL Precipitation Buffer (N4). Mix immediately by inverting the tube, or for large pellets, vigorously shaking the tube, until the mixture is homogeneous. Do not vortex. Centrifuge the lysate at >12,000 × *g* for 10 minutes.
5	**Bind**	Load the supernatant from step 4 onto a spin column in a 2-mL wash tube. Centrifuge the column at 12,000 × *g* for 1 minute. Discard the flowthrough and place the column back into the wash tube.
6	**Wash (Optional)**	(Recommended for endA+ strains). Add 500 µL Wash Buffer (W10) with ethanol to the column. Incubate the column for 1 minute at room temperature. Centrifuge the column at 12,000 × g for 1 minute. Discard the flowthrough and place column back into the wash tube.
7	**Wash and Remove Ethanol**	Add 700 µL Wash Buffer (W9) with ethanol to the column. Centrifuge the column at 12,000 × *g* for 1 minute. Discard the flowthrough and place the column into the wash tube. Centrifuge the column at 12,000 × *g* for 1 minute. Discard the wash tube with the flowthrough.
8	**Elute**	Place the Spin Column in a clean 1.5-mL elution tube. Add 75 µL of preheated TE Buffer (TE) to the center of the column. Incubate the column for 1 minute at room temperature.
9	**Recover**	Centrifuge the column at 12,000 × *g* for 2 minutes. The elution tube contains the purified plasmid DNA. Discard the column. Store plasmid DNA at 4°C (short term) or store the DNA in aliquots at –20°C (long term).

Miniprep plasmid isolation protocol (vacuum)

Follow this procedure to purify plasmid DNA using a vacuum manifold. Use a microcentrifuge, capable of centrifuging at >12,000 × *g*, a vacuum manifold, and a vacuum source. Follow the supplier's instructions to set up your vacuum manifold.

- Preheat an aliquot of TE Buffer (TE) to 65–70°C for eluting DNA. Heating is optional for eluting 1–30 kb plasmid DNA but is recommended for eluting DNA >30 kb.
- **Ensure the bag containing the PureLink™ Quick Spin Columns is closed tightly after each use.**
- **Caution: Buffers contain hazardous reagents. Use caution when handling buffers.**

Steps		Procedure Details
1	**Harvest**	Centrifuge 1–5 mL of the overnight LB-culture. (Use 1–2 × 10^9 *E. coli* cells for each sample.) Remove all medium.
2	**Resuspend**	Add 250 µL Resuspension Buffer (R3) with RNase A to the cell pellet and resuspend the pellet until it is homogeneous.
3	**Lyse**	Add 250 µL Lysis Buffer (L7). Mix gently by inverting the capped tube until the mixture is homogeneous. Do not vortex. Incubate the tube at room temperature for 5 minutes.
4	**Precipitate**	Add 350 µL Precipitation Buffer (N4). Mix immediately by inverting the tube, or for large pellets, vigorously shaking the tube, until the mixture is homogeneous. Do not vortex. Centrifuge the lysate at >12,000 × *g* for 10 minutes.
5	**Bind**	Remove the spin column from the wash tube (retain tube for later use) and attach the spin column to the luer extension and transfer the lysate from step 4. Apply vacuum. After all of the supernatant has passed through the column, turn off the vacuum.
6	**Wash (Optional)**	(Recommended for endA+ strains). Add 500 µL Wash Buffer (W10) with ethanol to the column. Incubate the column for 1 minute at room temperature. Apply vacuum. After all of the liquid has passed through the column, turn off the vacuum.
7	**Wash**	Add 700 µL Wash Buffer (W9) with ethanol to the column. Apply vacuum. After the liquid has passed through the column, turn off the vacuum.
8	**Remove Ethanol**	Place the column into a 2-mL wash tube. Centrifuge the column at 12,000 × *g* for 1 minute. Discard the wash tube and flowthrough.
9	**Elute**	Place the spin column in a clean 1.5-mL elution tube. Add 75 µL of preheated TE Buffer (TE) to the center of the column. Incubate the column for 1 minute at room temperature.
10	**Recover**	Centrifuge the column at 12,000 × *g* for 2 minutes. The elution tube contains the purified plasmid DNA. Discard the column. Store plasmid DNA at 4°C (short term) or store the DNA in aliquots at –20°C (long term).