Troubleshooting

Refer to the table below to troubleshoot your experiments with the PureLink™ HiPure Precipitator. For troubleshooting plasmid DNA purification, refer to the manual supplied with your plasmid purification kit.

Problem	Cause	Solution
Low or no DNA yield	Incomplete DNA precipitation	• Ensure the eluted plasmid DNA is in high salt buffer and be sure to add isopropanol to the eluted plasmid DNA prior to loading on the precipitator. • Ensure the precipitator membrane is completely dry following the ethanol wash by pushing air through the membrane twice.
	Large plasmid size	The precipitator is recommended for use with up to 40 kb plasmids.
	Incorrect elution parameters used	• Use the recommended elution volumes for the midiprep and maxiprep plasmid DNA. Using lower than the recommended elution buffer volume will not completely wet the entire membrane resulting in decreased yields. • Use warm buffer (heated to 60ºC) to elute plasmid DNA.
	Precipitator membrane damaged resulting in leaks	• Attach the precipitator to the syringe nozzle using the luer lock mechanism without applying excessive force. • Prior to removing the plunger from the syringe, always remove the PureLink™ HiPure Precipitator to avoid damaging the membrane. • Do not apply excessive pressure while pushing the solution through the PureLink™ HiPure Precipitator.
Precipitator is clogged	Too much DNA applied	Do not load eluate from several anion exchange columns onto the PureLink™ HiPure Precipitator.
	DNA precipitated with ethanol instead of isopropanol	Ethanol-precipitated DNA consists of fine particles that may clog the precipitator. Always use isopropanol to precipitate plasmid DNA.
Inhibition of downstream enzymatic reactions	Presence of ethanol in purified DNA	• Remove the ethanol by air-drying the membrane as described in the protocol. • Blot any ethanol droplets on the nozzle using a paper towel prior to elution.

Accessory Products

The table below lists additional products available from Invitrogen that may be used with the PureLink™ HiPure Precipitator Module. For more information, visit www.invitrogen.com or contact Technical Support.

Product	Quantity	Catalog no.
PureLink™ HiPure Filter Midiprep Kit	25 preps	K2100-14
	50 preps	K2100-15
PureLink™ HiPure Filter Maxiprep Kit	10 preps	K2100-16
	25 preps	K2100-17
PureLink™ HiPure Filter and Precipitator Maxiprep Kit	10 preps	K2100-26
	25 preps	K2100-27
PureLink™ HiPure Midiprep Kit	25 preps	K2100-04
	50 preps	K2100-05
PureLink™ HiPure Maxiprep Kit	10 preps	K2100-06
	25 preps	K2100-07
PureLink™ Nucleic Acid Purification Rack	1 each	K2100-13
Quant-iT™ DNA Assay Kit, High Sensitivity	1000 assays	Q33120
Quant-iT™ DNA Assay Kit, Broad-Range	1000 assays	Q33130
Quant-iT™ PicoGreen® dsDNA Assay	1 kit	P7589
Qubit™ Fluorometer	1 each	Q32857

PureLink™ HiPure Precipitator Module

Catalog No.	Quantity	Store at room temperature
K2100-21	10 Prep	
K2100-22	25 Prep	

Contents and Storage

The components included with the PureLink™ HiPure Precipitator Module are listed below. Sufficient reagents are included to process 10 samples (K2100-21) or 25 samples (K2100-22). Upon receipt, **store all components at room temperature**.

Components	K2100-21	K2100-22
PureLink™ HiPure Precipitator	10	25
Syringe, 5 ml	10	25
Syringe, 30 ml	10	25

Description

The PureLink™ HiPure Precipitator allows fast, simple, and efficient desalting and concentration of plasmid DNA isolated using anion-exchange chromatography. The traditional method for DNA precipitation of anion exchange isolated plasmid DNA involves isopropanol precipitation followed by centrifugation and drying of DNA which is time consuming and labor intensive. The use of the PureLink™ HiPure Precipitator allows plasmid DNA concentration within 5 minutes, eliminates the need for centrifugation, and reduces the risk of losing the DNA pellet during supernatant removal. The recovery is >80% and resulting plasmid DNA is ready to use in the most challenging applications such as transfection.

System Overview

Following plasmid DNA elution using anion exchange columns such as PureLink™ HiPure columns, the plasmid DNA is precipitated with isopropanol and applied to the PureLink™ HiPure Precipitator using a large syringe. The PureLink™ HiPure Precipitator traps the precipitated DNA on the membrane. After subsequent washing with 70% ethanol and a drying step, the plasmid DNA is eluted from the PureLink™ HiPure Precipitator with TE buffer or water into a microcentrifuge tube. The entire protocol is complete in ~5 minutes.

Specifications

Membrane Capacity:	Up to 2000 µg
Loading Volume:	Up to 30 ml (when attached to the 30 ml syringe)
Elution Volume:	0.5-1 ml (Midi scale) or 0.75-1 ml (Maxi scale)
Recovery:	80-99% (depends on elution volume)
Yield:	Up to 200 µg (Midi scale); up to 850 µg (Maxi scale)
Plasmid Size:	Up to 40 kb
Dead Volume:	60 µl
Material:	Polypropylene
Membrane Material:	Proprietary
Syringe Material:	Polypropylene
Inlet/Outlet Connections:	Female luer lock (inlet) and male slip luer (outlet)
Flow Rate:	145 ml/min
Maximum Operating Pressure:	60 psi
Chemical Compatibility:	Resistant to alcohols, organic solvents, acids, and bases
Processing Time:	~5 minutes

Compatibility

The PureLink™ HiPure Precipitator **is** compatible with the following plasmid purification kits:

- Maxiprep plasmid purification kits (PureLink™ HiPure Plasmid Filter Maxiprep Kit, PureLink™ HiPure Plasmid Maxiprep Kit available from Invitrogen, page 4, or equivalent anion exchange plasmid purification kits)
- Midiprep plasmid purification kits (PureLink™ HiPure Plasmid Filter Midiprep Kit, PureLink™ HiPure Plasmid Midiprep Kit available from Invitrogen, page 4, or equivalent anion exchange plasmid purification kits)

The PureLink™ HiPure Precipitator **is not** compatible with miniprep, megaprep, or gigaprep plasmid purification kits.

The PureLink™ HiPure Precipitator is designed to be used with the PureLink™ Nucleic Acid Purification Rack, or equivalent.

Product Qualification

The PureLink™ HiPure Precipitator is qualified to ensure the membrane material, filter housing, bed volume, inlet/outlet connections, flow rate, and operating pressure meet the set specifications.

Part no. K2100.pps Rev B. Date: 09 Aug 2007

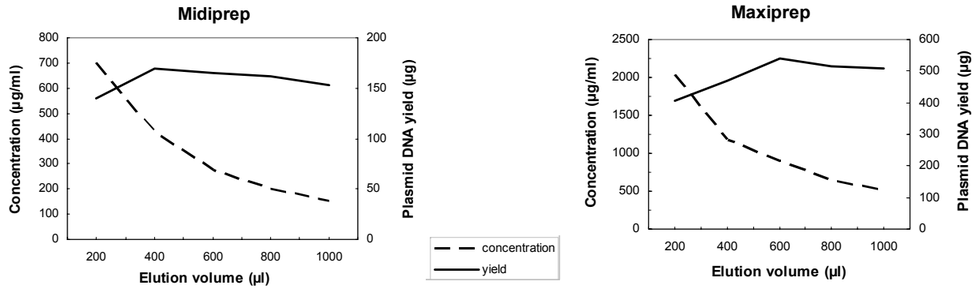
Important Guidelines

- Prior to removing the plunger from the syringe, always remove the PureLink™ HiPure Precipitator to avoid damaging the membrane.
- Do not apply excessive pressure while pushing the solution through the PureLink™ HiPure Precipitator, as too much pressure may detach the precipitator from the syringe. Use the PureLink™ Nucleic Acid Rack to assist with DNA precipitation (Figure 2).
- Attach the precipitator to the syringe properly using the luer lock mechanism to avoid the detachment of precipitator during sample processing.
- Always use proper aseptic techniques when working with DNA and use only sterile, DNase-free tips and tubes to prevent DNase contamination.
- Review the information below on **Elution Parameters** to obtain optimal DNA yield and concentration to suit your needs.

Elution Parameters

Elution Buffer Volume

Plasmid DNA is eluted in 0.5- 1 ml (midiprep plasmid DNA) or 0.75-1 ml (maxiprep plasmid DNA) of TE buffer. You can change the volume of elution buffer to obtain plasmid DNA in the desired final concentration. Use the graphs shown below to determine the most appropriate elution conditions for your application.



For increased DNA yield, use a higher volume of elution buffer. For increased DNA concentration, use a lower volume of elution buffer.

Elution Buffer

The plasmid DNA is eluted using TE buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA). Alternatively, Tris Buffer (10 mM Tris-HCl, pH 7.5) or sterile water can be used, if EDTA inhibits downstream reactions.

Number of Elutions

The first elution usually recovers ~90% of plasmid DNA. To maximize plasmid DNA recovery, you may perform a second elution by transferring the entire volume of eluate from the first elution back onto the syringe.

Elution Buffer Temperature

The Elution Buffer (TE) is recommended for use at room temperature. However, pre-warming the Elution Buffer to 60°C may increase the DNA yield by up to 10-15%, especially for eluting midiprep plasmid DNA or when plasmid copy number is low.

DNA Precipitation

Following plasmid DNA elution using anion exchange column, precipitate the plasmid DNA with isopropanol as follows and then proceed to **Using the PureLink™ HiPure Precipitator** to process the DNA precipitate:

- For PureLink™ HiPure Plasmid Filter Maxiprep and PureLink™ HiPure Plasmid Maxiprep Kit, add 10.5 ml isopropanol to the eluted DNA (15 ml). After mixing, incubate the DNA-isopropanol mixture for 2 minutes at room temperature.
- For PureLink™ HiPure Plasmid Filter Midiprep and PureLink™ HiPure Plasmid Midiprep Kit, add 3.5 ml isopropanol to the eluted DNA (5 ml). After mixing, incubate the DNA-isopropanol mixture for 2 minutes at room temperature.
- For equivalent anion exchange plasmid purification kits, refer to the manual supplied with the kit for DNA precipitation (generally, plasmid DNA is eluted in a high salt buffer, e.g.,100 mM Tris-HCl, pH 8.5 with 1.25 M NaCl and is mixed and precipitated with 0.7 volumes of isopropanol). After mixing, incubate the DNA-isopropanol mixture for 2 minutes at room temperature.

Materials Needed

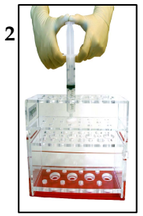
- Plasmid DNA purified using a midiprep or maxiprep purification kit (anion exchange chromatography)
- Isopropanol (room temperature)
- 70% ethanol
- TE Buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA), or Tris Buffer (10 mM Tris-HCl, pH 7.5), or sterile water
- DNase-free, pipette tips and microcentrifuge tubes

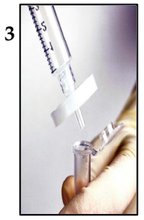
Using the PureLink™ HiPure Precipitator

1. Remove a 30 ml syringe (supplied with the module) from the package and remove the plunger from the syringe.
2. Attach the PureLink™ HiPure Precipitator through the luer lock inlet to the syringe nozzle (Figure 1) without using excessive force. Ensure the precipitator is properly attached to the syringe.
3. Use the PureLink™ Nucleic Acid Purification Rack to assist with precipitation (Figure 2), *or*, load the precipitated DNA mixture into the syringe, place the precipitator over a waste container, and insert the plunger. Push the plunger slowly to pass the DNA mixture through the PureLink™ HiPure Precipitator using constant force (figure 2). Discard the flow through.
4. Detach the PureLink™ HiPure Precipitator from the syringe, remove the plunger, and reattach the PureLink™ HiPure Precipitator to the syringe nozzle.

   Note: To prevent damaging the membrane, **do not** remove the plunger from the syringe while the precipitator is attached to the syringe.
5. To wash the DNA precipitate, add 3-5 ml 70% ethanol to the syringe, insert the plunger, and place the precipitator over a waste container. Push the plunger to pass the ethanol through the PureLink™ HiPure Precipitator.
6. Detach the PureLink™ HiPure Precipitator from the syringe, remove the plunger, and reattach the PureLink™ HiPure Precipitator to the syringe nozzle. Insert and push the plunger to pass air through the precipitator for drying the membrane.
7. Repeat Step 6 at least once.
8. Blot any ethanol droplets on the PureLink™ HiPure Precipitator nozzle with paper towel.
9. Detach the PureLink™ HiPure Precipitator from the 30 ml syringe and discard the 30 ml syringe.
10. Remove a 5 ml syringe (supplied with the module) from the package and remove plunger from the syringe. Attach the PureLink™ HiPure Precipitator to the 5 ml syringe nozzle.
11. To elute the plasmid DNA, add these recommended amounts of TE buffer to the syringe, insert the plunger, and place the precipitator over a clean, sterile microcentrifuge tube (see page 2 for elution parameters). Push the plunger to elute the plasmid DNA (Figure 3).
    - For Maxiprep plasmid DNA, use 0.75-1.0 ml buffer for elution
    - For Midiprep plasmid DNA, use 0.5-1.0 ml buffer for elution

    Note: Using warm TE (heated to 60ºC) improves recovery for midiprep plasmid DNA but not significantly for maxiprep plasmid DNA.
12. *Optional:* To perform a second elution, detach the PureLink™ HiPure Precipitator from the syringe, remove the plunger, and reattach the PureLink™ HiPure Precipitator to the syringe nozzle. Load the entire volume of eluate from the first elution back onto the syringe. Insert and push the plunger to perform a second elution of plasmid DNA into a microcentrifuge tube.
13. Store purified DNA at 4°C for immediate use or aliquot the DNA and store at –20°C for long-term storage. Avoid repeated freeze-thawing of DNA. Discard the PureLink™ HiPure Precipitator and syringes. **Do not** reuse the PureLink™ HiPure Precipitator or syringes.





DNA Quantitation

Perform DNA quantitation using UV absorbance at 260 nm or Quant-iT™ Kits.

UV Absorbance

1. Prepare a dilution of the DNA solution. Mix well. Measure the absorbance at 260 nm (A_{260}) of the dilution in a spectrophotometer (using a cuvette with an optical path length of 1 cm) blanked against the dilution buffer.
2. Calculate the concentration of DNA using the formula:

   DNA (µg/ml) = A_{260} × 50 × dilution factor

   For DNA, A_{260} = 1 for a 50 µg/ml solution measured in a cuvette with an optical path length of 1 cm.

Quant-iT™ Kits

Quant-iT™ Kits from Invitrogen provide a rapid, sensitive, and specific fluorescent method for dsDNA quantitation. Each kit contains a state-of-the-art quantitation reagent and a pre-made buffer to allow fluorescent DNA quantitation using standard fluorescent microplate readers/fluorometers or the Qubit™ Fluorometer.