

Hurricane Miniprep Kit PROTOCOL

Description: The Hurricane Miniprep Kit is designed for purification of up to 25 ug of high purity plasmid DNA from a starting volume of 2-5 ml of bacterial culture. The kit produces high quality plasmid DNA suitable for many applications such as restriction digestion, PCR, in vitro transcription, probe synthesis and DNA sequencing. The principle of the kit is based on the modified alkaline lysis technique for plasmid DNA purification.

Important Benefits:

- Fast and simple 20 minute protocol
- Column wash is carried out by 70% ethanol. Other kits use wash buffers containing salts which can inhibit downstream enzymatic applications
- Purified plasmid DNA is directly eluted into TE buffer or water at the final step.

Component List

1) Buffer A

ADD RNASE PRIOR TO USE

Dissolve RNase with 1 ml of Buffer A, re-suspend by briefly vortexing and spin down in a microfuge. Add the contents to Buffer A and mix well before use. Store the solution at 4 °C after the addition of RNase A.

2) Buffer B

Store the solution at room temperature. If the solution is cloudy (due to precipitation), warm the solution at 37 °C for 10 min. and mix the content before use. Store at room temperature.

3) Buffer C

Store the solution at room temperature. If the solution is not clear due to precipitation, warm the solution at 37 °C for 10 min. and mix the content before use. Store at room temperature.

4) RNase (one vial)

Briefly spin down the contents before use. Dissolve RNase with 1 ml of buffer 1. Add the contents to Buffer A and mix well before using the buffer.

5) DNA binding column unit (250 units)

6) Sterile water

Store at room temperature.

7) 1.5 ml collection tubes (250 tubes) for plasmid DNA elution

8) Protocol

Additional Materials and Equipment Required.

- Table top centrifuge capable of 12,000 rpm (10,000 x g)
- Heat Block or water bath set to 65-70° C
- 1.5mL tubes
- 70% Ethanol, Molecular Biology Grade
- Spectrophotometer for DNA quantification

Plasmid DNA Purification Protocol

Check that RNase has been added to Buffer A prior to beginning the protocol

1. Inoculate 2-5 ml LB containing appropriate antibiotic in a 15 ml tube with an *E. Coli* colony containing the desired plasmid. Grow the culture at 37 °C for 12- 16 hours with vigorous shaking (200-300 rpm).
 - For cells grown in LB broth harvest at a cell density of OD 595nm <1.2
 - For cells grown in TB broth harvest at a cell density of OD 595nm <1.6
2. Transfer the culture to a 1.5 ml centrifuge tube (not included). Pellet down the bacteria by centrifugation for 1 minute at 12,000 rpm at room temperature. Discard supernatant as much as possible. Repeat this step if more culture is needed.

Be sure to add RNase into Buffer A before use (see component list for details)

3. Re-suspend the bacterial pellet in **250 ul** of **Buffer A** by pipetting.
 - Complete re-suspension of bacteria is critical for high yield.
4. Add **250 ul** of **Buffer B**. Close the cap and mix the tube gently and thoroughly by inverting the tube 10 times. The mixture should become clear and viscous. **Do Not Vortex!**
 - **Vortexing shears genomic DNA and leads to genomic DNA contamination.**
5. Add **325 ul** of **Buffer C** to the tube. Gently mix the solution by inverting the tube 10 times. White precipitation should appear after mixing. **Do Not Vortex!**
6. Centrifuge the mixture for 10 minutes at 12,000 rpm at room temperature.
7. Carefully transfer the supernatant to the **DNA Binding Column Unit**. Centrifuge the column for 30 seconds to 1 minute at 12,000 rpm at room temperature to bind the DNA. Carefully remove the DNA Binding Column from the unit and discard the liquid from the collection tube. Reassemble the DNA binding column unit.
8. Add **750 ul** of **70% ethanol** to the DNA binding column unit. Centrifuge the unit at 12,000 rpm for 1 minute at room temperature. Carefully remove the DNA binding column from the unit and discard the pass through as much as possible from the collection tube with a P1000 pipetman. Reassemble the DNA binding column unit.
9. **OPTIONAL STEP (Recommended if DNA is intended for Sequencing):** Repeat step 8 once.
 - This step will ensure complete removal of contaminating salts which may inhibit Sequencing reactions.

10. Centrifuge the unit for an additional 3 min. at 12,000 rpm at room temperature.

11. OPTIONAL STEP

(*THIS STEP MUST BE PERFORMED IF DNA IS INTENDED FOR SEQUENCING***)**

Carefully remove the DNA binding column from the unit and discard the pass through as much as possible from the collection tube with a P1000 pipetman. Reassemble the DNA binding column unit. Centrifuge the unit for an additional 5 minutes at 12,000 rpm at room temperature.

- This step is important to ensure complete removal of Ethanol which can inhibit DNA sequencing reaction.

12. Transfer the DNA binding column to a new 1.5 ml collection tube (included). Add **50 ul** of preheated (65 - 70 °C) **Sterile Water** (included) to the center of the DNA binding column and let it stand at room temperature for 1 minute. Elute the plasmid DNA by centrifuging the unit for 1 minute at 12,000 rpm at room temperature.

Note: Sterile water is recommended by most sequencing facilities, you may choose to elute with TE if that better suits your application. TE is not included in this kit.

13. Transfer the eluted plasmid DNA from the collecting tube to a new 1.5 ml tube (not included) and store the DNA at 4 °C.

Note: For short term storage (up to 2 weeks) of DNA eluted in sterile water, 4 °C is recommended. For long term storage -20 °C is best.

Troubleshooting

Q: Low yield

1. Too many cells and not enough buffers to lyse cells completely. After resuspension of cell pellet in buffer A (step 3), the final total volume should not exceed 300 ul. If the volume exceeds 300 ul, double the volume of Buffer A, B and C in the purification process to ensure complete lysis.
2. Working with low copy plasmid. Harvest twice the amount of cell culture. Growing cells rich medium such as *Terrific Broth* can increase the density of cells. Remember to use twice the volume of buffer A, B and C in the purification process to ensure complete lysis.
3. Make sure the cells are re-suspended in buffer A (step 3) completely. Incomplete re-suspension of cells decreases the efficiency of lyses.
4. Make sure there is no precipitation in buffer B and C. Precipitation in these buffers decreases the efficiency of lyses. Warm the solutions at 37 °C for 10 min. and Vortex or shake well to re-dissolve the precipitants if necessary.

Q: Contamination of high molecular weight chromosomal DNA

1. During step 4 and 5, samples should not be vortexed or shaken vigorously. Also step 4 (lyses step) should not excess more than 10 min. Both can cause shearing of the genomic DNA and lead to high molecular weight chromosomal DNA contamination.

Q: Sample contains RNA

1. Too many cells were harvested. After addition of Buffer B (step 4), let the sample stands for additional 2-5 min. after mixing. If RNA problem persists, use twice amount of Buffer A, B and C in the purification process.
2. RNase activity is weakened. Buffer A with RNase A should be stored at 4°C to maintain its full activity. The full activity of RNase in Buffer A can be maintained for 6 – 12 months if stored at 4°C. Add more RNase A (100 µg/ml) to buffer A if the activity of RNase A is lost.

Q: Sample floats upon loading in agarose gel

1. Sample contains ethanol from the washing step. Make sure to follow steps 8-11 closely. If problem persists, spin the unit for 10 min. instead of 3 min. as recommended in step 10.

Q: O.D. 260/280 ratio above 2.2: The kit normally produces plasmid DNA with O.D. ratio between 1.9 to 2.2. If the ratio is above 2.2

1. Washing (steps 8-11) was not completed. Repeat steps 8-11 one more time.
2. Sample contains ethanol from the washing step. Make sure steps 8-11 are followed closely. If problem persists, spin the unit for 10 min. instead of 3 min. as recommended in step 10.

Appendix.

A. Culture Media recipes

LB broth:

10 g of Tryptone; 5 g of yeast extract; 5 g of NaCl

Adjust the pH of the medium to 7.4 with NaOH.

Add water to the medium to a final volume of 1000 ml.

Sterilize the medium by autoclaving. Cool down the temperature of the culture before inoculating.