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Hurricane Maxi Prep Kit
PROTOCOL

Description: The Hurricane Maxi Prep Kit is designed for purification of up to 1.2 mg of high purity plasmid DNA from a starting volume of 200-300 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.

Important Benefits:

• The procedure is simple and can be completed in about one hour

• 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 in the final step. Unlike other plasmid purification kits the Hurricane Maxi Prep Kit does not require alcohol precipitation and subsequent DNA re-suspension

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Component List

1) Solution 1(130 ml) (30 m in 2 prep trial kits)

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

2) Solution 2 (130 ml) (30 ml in 2 prep trial kits)

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 contents by gently inverting before use. Store at room temperature.

3) Solution 3 (180 ml) (40 ml in 2 prep trial kits)

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 contents by inverting before use. Store at room temperature.

4) RNase (one vial)

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

5) DNA binding column unit (10 units) (2 units in 2 prep trial kit)

6) TE (30 ml) (12 ml in 2 prep trial kit)

Store at room temperature.

7) 50 ml collection tubes for plasmid DNA elution (10 tubes) (2 tubes in 2 prep trial kit)

8) Protocol

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Additional Materials and Equipment Required.

- High speed centrifuge that is able to reach 13,000 rpm (14,000 - 18,000 x g) with appropriate rotor to hold 50 ml high-speed centrifuge tubes.
- Low speed centrifuge with swing-bucket rotor (preferred) or fixed-angle rotor that can hold standard 50 ml centrifuge tubes, and 250 ml or 500ml centrifuge bottles.
- Heat block or water bath for heating TE Buffer or sterile water to 65 - 70°C.
- 50 ml ***high speed*** centrifuge tubes (inferior tubes may shatter or crack).
- 50 ml disposable centrifuge tubes.
- 250 ml or 500ml centrifuge bottles for pelleting bacteria from culture.
- 70% ethanol (molecular biology grade).
- Sterile water for plasmid DNA elution (TE is included, sterile water may be substituted if desired)
- 1.5 ml tubes for storing purified plasmid DNA.
- Spectrophotometer for DNA quantification.

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Plasmid DNA Purification Protocol

Check that RNase has been added to Solution 1 prior to beginning the protocol

1. Inoculate 200-300 ml LB containing appropriate antibiotic in a 1 liter flask with 1.5 ml of an overnight culture of *E. Coli* 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.
   - Culture may be grown from a single colony but might require more time to reach desired density.

1. Transfer the culture to a 250 ml or 500ml centrifuge bottle (not included). Pellet down the bacteria by centrifugation for 10 min. at 5,000 x g at room temperature.

   Be sure to add RNase into Solution 1 before use (see component list for details)

3. Re-suspend the bacterial pellet in **10 ml** of **Solution 1** by pipetting.
   - Complete re-suspension of bacteria is critical for high yield.

4. Transfer the suspension to a **50 ml Disposable Centrifuge Tube** (not included). Add **10 ml** of **Solution 2**. Close the cap and mix the tube gently and thoroughly by inverting the tube 10 times. Let it stand at room temperature for 5 min. The mixture should become clear and viscous. Do Not Vortex!
   - Vortexing shears genomic DNA and leads to genomic DNA contamination. Incubation is crucial for complete RNase digestion.

5. Add **13 ml** of **Solution 3** to the tube. Gently mix the solution by inverting the tube 10 times. White precipitation should appear after mixing. Do not vortex!

   *** Note:** Steps 6 - 9 can be performed at room temperature. Results will be improved if steps 6 - 9 are carried out at 4 °C; the yield of super-coiled plasmid DNA will significantly increase.

6. Transfer the mixture directly to a **High Speed Centrifuge Tube** (not included) and centrifuge the mixture for 10 min. at 14,000 - 18,000 x g at room temperature or 4 °C*. If the high speed centrifuge is not available the sample can be spun longer at lower g force, e.g. 20 min. at 9,000 x g.

7. Carefully transfer the supernatant to a **50 ml Disposable Centrifuge Tube** (not included). Keep the tube at room temperature or 4 °C (on ice)*.

8. Transfer half of the supernatant to the **DNA Binding Column Unit** and centrifuge the column for 5 min. at 5,000 x g at room temperature or 4 °C* 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.

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9. Repeat step 8 for the remaining supernatant.

10. Add **20 ml** of 70% ethanol to the **DNA Binding Column Unit** and centrifuge the column at 5,000 x g for 5 min. at room temperature or 4 °C*. Carefully remove the DNA Binding Column from the unit and discard the liquid in the collection tube. Reassemble the DNA Binding Column Unit.

11. Repeat step 10. Use a P1000 pipetman to remove liquid in the collection tube as much as possible.

12. Centrifuge the unit for 10 min. at 5,000 x g at room temperature.
    - This step removes remaining Ethanol.

13. **Optional Step**

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

    Discard the liquid in the collection tube as much as possible with a P1000 pipetman. Reassemble the DNA binding column unit. Centrifuge the unit for an additional 15 min. at 5,000 x g at room temperature. This step ensures that no ethanol remains in the sample.

14. Open the cap of the unit and let the unit stand at room temperature for 10 min. to dry any leftover ethanol.

15. Carefully transfer the **DNA Binding Column** into a new **50 ml Collection Tube** (included). Avoid the DNA binding column contacting any leftover liquid at the bottom of the old collection tube during transfer. Add **1 ml** of preheated (65 - 70 °C) **TE Buffer** or sterile water (not included) to the center of the DNA binding column and let it stand at room temperature for 1 min. Elute the plasmid DNA by centrifuging the unit for 5 min. at 5000 x g at room temperature

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

(Please Continue)

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16. **Optional Step (recommended to generate maximum total yield of DNA)**
    Add **1 ml** of pre-heated (65 - 70 °C) **TE Buffer** (included) or sterile water (not included) to the center of the DNA binding column and let it stand at room temperature for 1 min. Elute the plasmid DNA by centrifuging the unit for 5 min. at 5000 x g at room temperature.

    NOTE: 65-70% of DNA is eluted from the column in the first elution step. If high concentration of plasmid DNA is desired DO NOT combine the first elution with the second elution. Use the first elution for applications requiring high DNA concentrations.If decreasing the concentration is not of concern, you may combine the first and second elution into a single tube.

17. Combined the two fractions of eluted DNA if needed and store the DNA at 4 °C if it is eluted with TE Buffer, or - 20 °C if it is eluted
    - 60 – 75% of plasmid DNA is eluted from the column from the first elution. If high concentration of plasmid DNA is desired do not combine the first elution with the second elution. Use the first elution Plasmid for applications requiring high DNA concentration.

STORAGE NOTE: For long term storage, DNA suspended in water needs to be stored frozen (-20°C) to prevent hydrolization. Repeat freezing and thawing of plasmid DNA in water may cause DNA degradation. Divide the purified plasmid DNA in water in small aliquots and store them at - 20 °C if repeat freezing and thawing can not be avoided.

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Troubleshooting

Q. Low yield

1. Too many cells and not enough solutions to lyse cells completely. After re-suspension of cell pellet in solution 1 (step 3), the final total volume should not exceed 11 ml. If the volume exceeds 11ml, double the volume of solution 1, 2 and 3 in the purification process to ensure complete lysis. When doubling the volume of solution 1,2, and 3, repeat steps 8 and 9.

2. Working with low copy plasmid. Harvest twice the amount of cell culture. Growing cells in rich medium such as *Terrific Broth* can increase the density of cells. Remember to use twice the volume of solution 1, 2 and 3 in the purification process to ensure complete lysis. When doubling the volume of solution 1,2, and 3, repeat steps 8 and 9.

3. Make sure the cells are re-suspended in solution 1 (step 3) completely. Incomplete re-suspension of cells decreases the efficiency of lysis.

4. Make sure there is no precipitation in solution 2 and 3. Precipitation in these solutions decreases the efficiency of lysis. Warm the solutions at 37 °C for 10 min. and Vortex or shake well to redissolve 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 (lysis 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 solution 2 (step 4), let the sample stands for additional 2-5 min. after mixing. If RNA problem persists, use twice amount of solution 1, 2 and 3 in the purification process.

2. RNase activity is weakened. Solution 1 with RNase A should be stored at 4°C to maintain its full activity. The full activity of RNase A in solution 1 can be maintained for 6 – 12 months if stored at 4°C. Add more RNase (100 μg/ml) to solution 1 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 10-14 closely. If problem persists, spin the unit for 20 min. instead of 10 min. as recommended in step 12.

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Q. O.D. ratio between 260 nm and 280 nm 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 10-14) was not completed. Repeat steps 10-14 one more time.

2. Sample contains ethanol from the washing step. Make sure steps 10-14 are followed closely. If problem persists, spin the unit for 20 min. instead of 10 min. as recommended in step 12.

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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.

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