MediRes Corp.

USER GUIDE **Bi2M-PlasMidi**

Plasmid Midi Kit

Product #: Bi2M-PlasMidi
Quantity: 10 reactions

Product Description

- Designed for rapid and cost-effectives medium-scale preparation of high quality plasmid DNA from recombinant *E.coli* cultures.
- Based on modified SDS-alkali lysis method and utilizes an exclusive silica-based membrane technology in the form of a convenient spin column. The kit utilizes proprietary glass fiber membrane for specific plasmid adsorption and a specialized buffer combination.
- Effectively extracts various endotoxin-free plasmids (≤ 0.1 EU/µg endotoxin content) from cultured bacteria.
- Typical plasmid yield ranges from 100 to 400 µg.
    - *The actual plasmid yield will depend on the plasmid copy number & culture density.*
- The purified plasmid DNA can be used in all molecular biology procedures such as conventional digestion with restriction enzymes, PCR, transformation and sequencing.

Storage

- Store RNaseA at -20 ℃. All other items can be stored at room temperature for up to 12 months. For longer storage, store at 4℃.
- After addition of RNase A, the resuspension solution **Buffer P1** should be stored at 4℃.

Kit Components

Buffer BL	25 mL
Buffer P1	30 mL (*note: add 600 µL **RNase A**, and store at 4℃)*
Buffer P2	30 mL (*note: seal tightly after each use*)
Buffer P3	30 mL (*note: pre-cool to 4 ^{0}C before use*)
Buffer ER	10 mL
Buffer ED	40 mL
Buffer PW	16 mL (*note: add 64 mL 100% ethanol per bottle before use*)
Buffer TE	12 mL (*note: pre-heat to 60℃ before use*)
RNase A	600 µL

Column Filters	10 columns
HiBind DNA Midi Column	10 columns
Collection Tube	10 tubes

Important Preparatory Notes (PLEASE READ CAREFULLY)

1. Prepare a 42°C water bath or heating module.
2. Add the entire **RNase A** provided to **Buffer P1** before use; it can then be stored at 4°C for up to 6 months.
3. Add **64 mL of anhydrous ethanol** to **Buffer PW** before use.
4. If **Buffer P2** has precipitated, heat it in a 37°C water bath for a few minutes until it becomes clear. Close the lid immediately after use to prevent prolonged exposure to air.
5. Pre-cool Buffer P3 at 4°C before use.
6. Always keep buffer bottles tightly closed and keep columns sealed in the enclosed zip-lock bag.
7. Proper laboratory safety practices should be employed, including the use of lab coats, gloves, and eye protection.

Protocol

1. **Column Preparation:** Add 2 mL of **Buffer BL** to the **HiBind DNA Midi Column**, centrifuge at **4,500×g** for **2 minutes** at **room temperature**. Discard the filtrate and return the HiBind DNA Midi Column to the Collection Tube. It is recommended to use the prepared column immediately.
2. **Bacterial Culture Harvest:** Harvest bacterial culture from 20-50 mL of fresh bacterial culture incubated for 12-16 h by centrifugation at **8,000 x g** for **5 min** at **room temperature**. Decant the supernatant and remove all remaining medium.

- *Note: For **high-copy-number plasmids**, do not process more than 50 mL of bacterial culture. If more than 50 mL, the Hi-Bind DNA Mini Column capacity will be exceeded and no increase in plasmid yield will be obtained.*
- *Note: For **low-copy-number plasmids**, it may be necessary to process larger volumes of bacterial culture (up to 100 mL) to recover sufficient quantity of DNA.*

3. **Resuspend Bacteria:** Add **2.5 mL** of **Buffer P1** (*note: ensure **RNase A** is added*) and thoroughly resuspend the bacterial pellet using a pipette or vortex. Ensure the bacteria are fully dispersed to optimize lysis and achieve high-quality plasmid extraction.
4. **Cell Lysis:** Add **2.5 mL** of **Buffer P2**, then gently invert the tube 8–10 times to lyse the cells completely. Avoid vigorous shaking and complete this step within **5 minutes**. The solution should become clear and viscous. If not, the culture density may be too high; consider reducing the amount of bacteria.
5. **Neutralization:** Add **2.5 mL** of **pre-chilled Buffer P3**, then gently invert the tube 10–12 times until a precipitate forms. Centrifuge at **8,000×g** for **15 minutes** at **room temperature**. Carefully pour the supernatant into the Column Filter, filter by pushing the handle, and collect the filtrate in a nuclease-free 15 mL centrifuge tube (not provided).
6. **Endotoxin Removal:** Add **750 µL** of **Buffer ER** and mix by inverting 3–5 times. Place the solution on **ice** for **10 minutes**, during which the solution should turn transparent blue.
7. **Incubate and Separate Layers:** Incubate at **42°C** for **5 minutes** until the solution becomes turbid. Centrifuge at **4,500×g** for **5 minutes** at **room temperature**. The solution will separate into a clear upper aqueous layer and a blue lower layer. Carefully transfer the upper layer to a new nuclease-free 15 mL centrifuge tube.
8. **Ethanol Precipitation:** Add **anhydrous ethanol** equal to **half the volume** of the collected solution. Mix by inverting and transfer to the **HiBind DNA Midi Column** (maximum 4 mL).

9. **Column Washing:** Centrifuge at **4,500×g** for **3 minutes** at **room temperature**. Discard the filtrate and return the **HiBind DNA Midi Column** to **the Collection Tube**. Repeat the loading step if necessary.
10. **First Wash with Buffer ED:** Add **3.5 mL** of **Buffer ED** to the **HiBind DNA Midi Column** and centrifuge at **4,500×g** for **3 minutes** at **room temperature**. Discard the filtrate and return the column to the **Collection Tube**.
11. **First Wash with Buffer PW:** Add **3.5 mL** of **Buffer PW** (*note: ensure anhydrous ethanol has been added*) to the **HiBind DNA Midi Column**, then centrifuge at **4,500×g** for **3 minutes** at **room temperature**. Discard the filtrate and return the column to the Collection Tube.
12. **Second Wash with Buffer PW:** Repeat Step 11.
13. **Final Spin:** Centrifuge at **4,500×g** for **10 minutes** at **room temperature**. Place the **HiBind DNA Midi Column** into a new nuclease-free 15 mL centrifuge tube. Open the lid and let it sit for **10 minutes** at **room temperature** to allow complete ethanol evaporation.
14. **Elution:** Add **100–500 µL** of **Buffer TE** or **endotoxin-free water** to the center of the column membrane and let it sit at **room temperature** for **5 minutes**. Centrifuge at **4,500×g** for **5 minutes**.
    - For higher plasmid yield, reapply the eluted solution to the **HiBind DNA Column**, allow it to sit for **5 minutes**, and centrifuge again at **4,500×g** for **5 minutes**.
15. **Storage:** Store the purified plasmid DNA at -20°C or or -80℃ for long-term preservation. Avoid repeated freeze-thaw cycles.

NOTES:

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