MediRes Corp.

USER GUIDE **Bi2M-UDNAXtract**

Universal DNA Purification and Gel Extraction Kit

Product #: Bi2M-UDNAXtract

Quantity: 100 reactions

Product Description

- Designed for rapid and cost-effective purification of DNA from TAE or TBE agarose gels, or recovering DNA form PCR products.
- Based on silica-based membrane technology in the form of a convenient spin column.
- Each column can recover up to 20 µg of DNA.
- Contains visual pH indicator for ensuring optimal reaction chemistry.
- The purified DNA can be used in all molecular biology procedures such as conventional digestion, cloning, PCR and sequencing.

Storage

- Store at room temperature for up to 12 months.

Kit Components

Buffer BL	50 mL
Buffer GL (Yellow)	60 mL
Buffer PW	2×15 mL
Elution Buffer	12 mL (*note: pre-heat to 60℃ before use*)
RNase A	300 µL
Bind DNA Mini Column	100 columns
Collection Tube	100 tubes

Important Preparatory Notes (PLEASE READ CAREFULLY)

1. If precipitate forms in the buffers during storage, solutions should be re-dissolved by incubating at 37℃, then cooled back down to 25℃ before use.
2. Add **60 mL 100% ethanol** to the **Buffer PW** and mark the bottle on the label.
3. Prepare water bath or heat block at 60℃.

4. Always keep buffer bottles tightly closed and keep columns sealed in the enclosed zip-lock bag.
5. Proper laboratory safety practices should be employed, including the use of lab coats, gloves, and eye protection.
6. (Optional step) Column balance:
    - Put the **Bind DNA Mini Columns** into the **Collection Tubes**, add **500 µL** solution **Buffer BL** to the adsorption column, centrifuge at 10,000 g for 1 min, remove the waste liquid in the **Collection Tubes**.
    - Return the **Bind DNA Mini Columns** to the **Collection Tube** (please use the column treated on the day).
    - Note: Addition of **Buffer BL** can improve the adsorption capacity, homogeneity and stability of the **Bind DNA Mini Columns**, and eliminate the influence of high temperature, humidity or other adverse environmental factors on the **Bind DNA Mini Columns**.

Gel Extraction Protocol

1. **Excising the gel slice:** After completing agarose gel electrophoresis, excise the area of the gel containing the desired DNA fragment using a clean, sharp razor blade. Minimize the amount of agarose surrounding the DNA fragment.
2. **Weigh** the gel slice containing the DNA fragment using a scale sensitive to 0.001 g. Place the gel slice into a **1.5-mL microcentrifuge tube** (for≤2% agarose gels) or a 5.0-mL microcentrifuge tube(for≥2% agarose gels).
    - *Note: The maximum amount of starting gel is **≤400 mg per tube**. If your gel slice exceeds 400 mg, cut the gel into smaller slices to ensure no one piece exceeds 400 mg. Place additional gel slices into separate microcentrifuge tubes. For this case, an additional **Bind DNA Mini Column** is required for each extra gel slice during the purification.*
3. **Dissolving the gel slice:** Place ≤400 mg of the excised gel containing DNA into microcentrifuge tube, add **equal volume** of **Buffer GL** to every 1 volume of gel (e.g., add 100 µL of Buffer GL for every 100 mg of gel).

4. Place the tube containing the gel slice and Buffer GL into a **60℃ water bath** or **heat block** and incubate at **60℃** for **10 min** (or longer, please see note below). Invert the tube every 3 min to ensure complete gel dissolution.
   - *Note: High concentration gels (＞2% agarose) or large gel slices may take longer than 10 min to dissolve.*
5. Add half gel volume of **isopropanol** to the dissolved gel slice. The best color of gel solution is faint yellow. If the color of the gel solution is red or violet, add 10 µL of **3 M sodium acetate solution** (pH 5.2) to adjust the gel solution to faint yellow.
6. Pipet the dissolved gel piece solution containing the DNA fragment into the center of a **Bind DNA Mini Column** inside a **Collection Tube**. Centrifuge the tube at 10,000 g for 1 min. Discard the flow-through and replace the Bind DNA Mini Column into the Collection Tube.
   - *Note: Do not load > 800 µL gel solution at one time. If the total volume exceeds 800 µL, the gel solution can be divided and applied with multiple centrifugation steps.*
7. Add **700 µL** of **Buffer PW** (please check whether 100% ethanol was added before use) to the **Bind DNA Mini Columns**.
   - *Note: If the recovered DNA is used for salt-sensitive experiments, such as flat end joining experiment or direct sequencing, let the tube stand for 5 min after adding Buffer PW.*
8. Centrifuge the tube at 10,000 g for 1 min. Discard the flow-through and replace the column into the Collection tube.
9. Add **700 µL** of **Buffer PW** to the **Bind DNA Mini Columns**. Centrifuge the tube at 10,000 g for 1 min. Discard the flow-through and replace the column into the Collection tube.
10. Centrifuge the tube again at 10,000 g for 3 min to remove residual Buffer PW and ethanol. **Discard** the Collection Tube and place the Bind DNA Mini Column into a **new 1.5-mL centrifuge tube**. Let the tube stand at room temperature for 5 min.
11. Add 30-50 µL **Elution Buffer** or ddH_2O to the center of the **Bind DNA Mini Column**. Incubate the tube at room temperature for 2 min. Centrifuge the tube at 10,000 g for 2 min to collect the purified DNA.
    - *Note: pre-heat **Elution Buffer** or **ddH_2O** to 60℃ before use.*

- *Note: take care not to contact the membrane with the pipette tip.*
- *Note: minimum elution volume is 20 µL.*

12. Store the purified DNA at 4℃ for immediate use or aliquot the DNA and store at -20℃ for long-term storage. Avoid repeated freezing and thawing of the DNA.

PCR Product Purification Protocol

1. Add **3 volume**s of **Buffer GL** to 1 volume of PCR reaction, mix well.
   - Note: Minimum PCR volume is 50 uL. Adjust with water.
   - Note: Maximum PCR volume is 100 µL.
2. Add **3/4 volume** of **isopropanol** to 1 volume of PCR reaction, mix well.
3. Add sample to the **Bind DNA Mini Column** (placed into the **Collection Tube**).
4. Centrifuge at room temperature at 10,000 g for 1 min.
5. Discard the flow-through and replace the **Bind DNA Mini Column** into the **Collection Tube**.
6. Add **700 µL** of **Buffer PW** (Please check whether 100% ethanol was added before use) to the **Bind DNA Mini Column**.
   - *Note: If the recovered DNA is used for salt-sensitive experiments, such as flat end joining experiment or direct sequencing, it is recommended to let the tube stand for 5 min after adding Buffer PW.*
7. Centrifuge at 10,000 g for 1 min. Discard the flow-through and place the **Bind DNA Mini Column** back into the **Collection Tube**.
8. Add **700 µL** of **Buffer PW** to the **Bind DNA Mini Column**. Centrifuge at 10,000 × g for 1 min.
9. Centrifuge the tube again at 10,000 g for 3 min to remove residual Buffer PW and ethanol. **Discard** the Collection Tube and place the Bind DNA Mini Column into a **new 1.5-mL centrifuge tube**. Let the tube stand at room temperature for 5 min.
10. Add 30-50 µL **Elution Buffer** or ddH_2O to the center of the **Bind DNA Mini Column**. Incubate the tube at room temperature for 2 min. Centrifuge the tube at 10,000 g for 2 min to collect the purified DNA.

- *Note: pre-heat **Elution Buffer** or **ddH_2O** to 60℃ before use.*
- *Note: take care not to contact the membrane with the pipette tip.*
- *Note: minimum elution volume is 20 µL.*

11. Store the purified DNA at 4℃ for immediate use or aliquot the DNA and store at -20℃ for long-term storage. Avoid repeated freezing and thawing of the DNA.

NOTES:

1. **DISCLAIMER**: TO THE EXTENT ALLOWED BY LAW, MEDIRES CORP. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.
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