MediRes Corp.

USER GUIDE **G3633-50T**

Genomic DNA Extraction Kit (Spin Column Based)

Product #: G3633-50T

Quantity: 50 reactions

Product Description

- Designed for rapid and cost-effective purification of genomic DNA from animal tissues, mammalian cells, microbial cells and whole blood.
- High purity genomic DNA extraction is achieved by combining a specially optimized lysis buffer with a proprietary silica-based membrane technology in the form of a convenient spin column.
- Rapidly extracts and purifies 10-30 µg of high purity genomic DNA from:
  - 2-30 mg of animal tissue
  - 10^6 to 10^7 freshly cultured cells
  - 5-100 µL of whole blood (including anticoagulant) with non-nucleated or nucleated red cells
- Genomic DNA obtained is ready for applications such as PCR, Southern blotting, RAPD, AFLP, RFLP, sequencing and other molecular experiments.

Kit Components – BAG #1 (store at room temperature)

Buffer GA	12 mL
Buffer GA2	25 mL x 2
Buffer GB	12 mL
Buffer PD	12 mL (*note: add 18 mL 100% ethanol per bottle before use*)
Buffer PW	24 mL (*note: add 56 mL 100% ethanol per bottle before use*)
Buffer TE	10 mL
DNA Spin Columns	50 columns
Collection Tubes	50 columns

Kit Components – BAG #2 (store at -20^{0}C)

Proteinase K	1 mL
RNase A	200 µL

Additional Required Reagents

100% Ethanol

Important Preparatory Notes (PLEASE READ CAREFULLY)

1. If precipitate forms in **Buffer GA, Buffer GA2** or **Buffer PD** during storage, solution should be re-dissolved by incubating at 37℃, then cooled back down to 25℃ before use.
2. Add **18 mL 100% ethanol** to the **Buffer PD** and mark the bottle on the label.
3. Add **56 mL 100% ethanol** to the **Buffer PW** and mark the bottle on the label.
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.

RNA Extraction Protocol

1. **Sample preparation:**

- **Animal tissue:** Transfer 2-30 mg of fresh or cryopreserved animal tissue to an appropriately sized Nuclease-free tube. Add two or three 4 mm stainless steel grinding beads (Cat# G0104) per sample. Immediately add **200 µL** of **Buffer GA**. Use an automated homogenizer (TissueLyser) to homogenize thoroughly (1 to 3 min @ 20-50 Hz). Let the sample stand at room temperature for 2 minutes.

- **Adherent cells:** Remove the growth medium from the cells and wash cells with 1×PBS buffer (pH 7.4). Harvest 10^6 to 10^7 cells and transfer to an appropriately sized Nuclease-free tube. Discard the supernatant. Immediately add **200 µL** of **Buffer GA** and mix well by vortex. Let the sample stand at room temperature for 2 minutes.

- **Non-adherent suspension cells**: Transfer 10^6 to 10^7 cells to an appropriately sized Nuclease-free tube and centrifuge at 1,000 x g for 5 minutes at 4°C to pellet. Discard the growth medium from the tube. Add

200 µL of **Buffer GA** and mix well by vortex. Let the sample stand at room temperature for 2 minutes.

- **Microbial cells**: Grow a fresh 10 mL overnight culture. After incubation, centrifuge the culture at 12,000 x g for 10 minutes to harvest the cells. Discard the supernatant and resuspend the pelleted cells in **1 mL** of **Buffer GA2** (cell pellet can be stored at -80^{0}C before addition of Buffer GA2 as a safe stopping point). Transfer the cell suspension to a 1.5 mL microcentrifuge tube containing 100 mg of 0.1 mm acid-washed glass grinding beads (Cat# G0310) for bacterial samples, and 100 mg of 0.5 mM acid-washed glass grinding beads (Cat# G0350) for yeast/fungal samples. Homogenize thoroughly using an automated homogenizer (TissueLyser) for 3 minutes at 30 Hz. Transfer 200 µL of the homogenized sample to a new 1.5 mL microcentrifuge tube and discard the remainder. Add **200 µL** of **Buffer GA** to the sample, mix well by vortexing, and let the sample stand at room temperature for 2 minutes.

- **Blood (non-nucleated erythrocytes)**: Transfer 50-100 µL whole blood of non-nucleated erythrocytes (including anticoagulant) to an appropriately sized Nuclease-free tube. Add **200 µL** of **Buffer GA** and mix well by vortex. Let the sample stand at room temperature for 2 minutes.

- **Blood (nucleated erythrocytes)**: Transfer 5-20 µL whole blood of nucleated erythrocytes (including anticoagulant) to an appropriately sized Nuclease-free tube. Add **200 µL** of **Buffer GA** and mix well by vortex. Let the sample stand at room temperature for 2 minutes.

2. <u>Preparation and Digestion:</u>

- Add **20 µL** of **Proteinase K** and **4 µL** of **RNase A** to the sample from **step 1**.
- Incubate at 56℃ for 30 minutes, mixing every 10 minutes to accelerate tissue lysis.
- <u>Note:</u> Extend incubation time for materials that are difficult to lyse.

3. **Centrifugation:**
   - Centrifuge at 12,000 x g for 2 minutes.
   - Transfer the supernatant to a nuclease-free centrifuge tube, avoiding the pellet and beads.

4. **Buffer Addition:**
   - Add **200 µL** of **Buffer GB** to the centrifuge tube, mix thoroughly, and incubate at 70°C for 10 minutes.

5. **Ethanol Addition:**
   - Add **200 µL** of **100% ethanol** to the centrifuge tube, mix well (precipitate may form after adding ethanol), and then centrifuge at 12,000 x g for 2 minutes.

6. **Supernatant Transfer:**
   - Place the **DNA Spin Columns** into the **Collection Tubes** and transfer all the mixture to the DNA Spin Columns, avoiding the pellet.

7. **DNA Binding via Initial Centrifugation:**
   - Centrifuge at 12,000 x g for 30 seconds.
   - Discard the flow-through and return the DNA Spin Columns to the Collection Tube.

8. **Wash Steps:**
   - Add **500 µL** of **Buffer PD** to the DNA Spin Columns, centrifuge at 12,000 x g for 30 seconds, discard the flow-through, and return the DNA Spin Columns to the Collection Tube.
   - Add **600 µL** of **Buffer PW** to the DNA Spin Columns, centrifuge at 12,000 x g for 30 seconds, discard the flow-through, and return the DNA Spin Columns to the Collection Tube.
   - **Repeat** the Buffer PW wash step.

9. Final Centrifugation:

- Centrifuge at 12,000 x g for 2 minutes to remove any residue.

10. Elution:

- Place the DNA Spin Columns into a **new 1.5 mL centrifuge tube** and let sample stand at room temperature for 5-10 minutes.
- Add **50-100 µL** of **Buffer TE** or nuclease-free water to the center of the membrane (note: pre-heating the Buffer TE or nuclease-free water to 65℃ can improve elution efficiency).
- Centrifuge at 12,000 x g for 2 minutes to collect DNA.
- Optional: for increased yield, re-add the flow-through to the center of the membrane, let it stand for 5 minutes at room temperature, and centrifuge again at 12,000 x g for 2 minutes.

11. Storage:

- Store the purified genomic at -80℃ for long-term storage. Avoid repeated freezing and thawing of the DNA.