TIANGEN®

TIANamp Micro DNA Kit

For isolation of genomic DNA from
small volumes of blood
dried blood spots
plasma and serum
mouthwash
hair follicles
tissues
microdissected tissues

www.tiangen.com/en

TIANamp Micro DNA Kit

(Spin Column)

Cat. no. GDP316

Kit Contents

Contents	GDP316 50 preps
Buffer GA	15 ml
Buffer GB	15 ml
Buffer GD	13 ml
Buffer PW	15 ml
Buffer TB	15 ml
Proteinase K	1 ml
Carrier RNA	310 μg
RNase-Free ddH_2O	1 ml
RNase-Free Spin Columns CR2	50
Collection Tubes 2 ml	50
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Storage

TIANamp Micro DNA Kit can be stored at room temperature (15-30°C) for up to 15 months without showing any reduction in performance and quality. If any precipitate forms in the buffers, it should be dissolved by warming the buffers at 37°C for 10 min before use. Stock solution of Carrier RNA should be stored at -30~-15°C.

Introduction

TIANamp Micro DNA Kit is based on silica membrane technology and provides special buffer system. Genomic DNA binds to the silica-membrane in the presence of high salt, while the contaminants pass through the column. After the membrane is thoroughly washed to remove any remaining contaminants, the pure DNA is eluted from the membrane with low salt buffer.

The kit is suitable for a wide range of sample materials such as small volumes of blood, dried blood spots, serum/plasma, tiny amount of tissues, mouthwash, hair follicles. Purified genomic DNA can directly serve as templates for PCR, restriction enzyme digestion, hybridization, *etc*.

Important Notes

1. Reagents to be supplied by user: Ethanol (96-100%), 1M DTT (for genomic DNA extraction from hair follicles)
2. Technical Index of TIANamp Micro DNA Kit

Maximum Capacity of TIANamp Spin Column CR2	700 µl
Minimal Elution Volume of Buffer TB	20 µl
Maximum Volume of Anti-coagulant Whole Blood (Mammalian)	100 µl
Maximum Amount of Animal Tissues	10 mg

3. If a precipitate has formed in Buffer GA or GB, please warm them at 37°C to dissolve the precipitate.
4. Equilibrate samples to room temperature (15-30°C).
5. Ensure that ethanol (96-100%) has been added to Buffer GD and Buffer PW as indicated on the tag of bottle at the first use.
6. For high yield of DNA, the kit supplies Carrier RNA. Direct analysis of the genomic DNA by PCR is recommended, since use of Carrier RNA results in errors in OD_{260}.

Preparation of Carrier RNA Stock Solution

At the first use of Carrier RNA, add 310 µl RNase-Free ddH_2O to the tube containing 310 µg lyophilized Carrier RNA to obtain a solution of 1 µg/µl. Dissolve the Carrier RNA thoroughly, divide it into conveniently sized aliquots, and store at -20°C. Do not freeze-thaw the aliquots of Carrier RNA more than 3 times.

Protocol:

<1> Genomic DNA from Small Volumes of Blood

Ensure that ethanol (96-100%) has been added to Buffer GD and PW according to the instructions at the first use.

1. Pipet 1-100 µl whole blood into a 1.5 ml microcentrifuge tube (not supplied).
2. Add Buffer GA to a final volume of 100 µl, if the blood sample is less than 100 µl.
3. Add 10 µl Proteinase K.

   Note: If RNA-free DNA is required, 5 µl RNaseA (100 mg/ml) (not provided) can be added. Vortex 15 sec and incubate for 5 min at room temperature (15-30°C).
4. Add 100 µl Buffer GB (If the initial volume of blood is lower than 10 µl, add 1 µl Carrier RNA Stock Solution (1 µg/µl, please refer to page 2 for preparation of Carrier RNA Stock Solution) to Buffer GB), close the lid, and mix by inverting the tube gently. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid. Incubate at 56°C for 10 min, and shake the 1.5 ml microcentrifuge tube gently during that time.

   Note: A white precipitate may form when Buffer GB is added. The precipitate will not interfere with the procedure and will dissolve during the heat incubation at 56°C. If the precipitate does not dissolve, it indicates that the cell is not completely lysed and may result in low yield of DNA and impurity in DNA.
5. Add 50 µl ethanol (96-100%) (If room temperature exceeds 25°C, cool the ethanol on ice before adding to the 1.5 ml microcentrifuge tube), close the lid, and mix by inverting the tube gently. Incubate for 3 min at room temperature. Briefly centrifuge to remove drops from inside the lid.
6. Carefully transfer the entire lysate from step 5 to the Spin Column CR2 (in a 2 ml Collection Tube) without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (13,400 × g) for 30 sec. Discard the flow-through and replace the Spin Column CR2 in the Collection Tube.

7. Carefully open the Spin Column CR2 and add 500 µl Buffer GD **(Ensure ethanol has been added before use)** without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and replace the Spin Column CR2 in the Collection Tube.
8. Carefully open the Spin Column CR2 and add 600 µl Buffer PW **(Ensure ethanol has been added before use)** without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and replace the Spin Column CR2 in the Collection Tube.
9. Repeat Step 8.
10. Replace the Spin Column CR2 in the Collection Tube, and centrifuge at 12,000 rpm (~13,400 × g) for 2 min. Discard the flow-through and place Spin Column CR2 in room temperature for 2-5 min to dry the membrane completely.

    Note: The residual ethanol of buffer PW may have some affection in downstream application.
11. Place the Spin Column CR2 in a clean 1.5 ml microcentrifuge tube (not provided) and apply 20-50 µl Buffer TB to the center of the membrane. Close the lid and incubate at room temperature for 2-5 min. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min.

    Note: The elution volume should not be less than 20 µl since smaller volume will affect recovery efficiency. For high yield of DNA, the flow-through containing DNA can be added to CR2 again. Incubate the column at room temperature for 2 min. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min. The pH value of eluted buffer has some influence in eluting; Buffer TB or distilled water (pH 7.0-8.5) is suggested to elute DNA. For long-term storage of DNA, eluting in Buffer TB and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.

Protocol:

<2> Isolation of Genomic DNA from Dried Blood Spots

Ensure that ethanol (96-100%) has been added to Buffer GD and Buffer PW according to the instructions at the first use.

1. Cut 3 × 3 mm sample from a dried blood spot and place it into a 1.5 ml microcentrifuge tube (not supplied).
2. Add 180 µl Buffer GA.
3. Add 20 µl Proteinase K and mix by inverting gently. Incubate at 56°C for 1 h and vortex the tube for 10 sec every 10 min to improve lysis.
4. Add 200 µl Buffer GB and 1 µl Carrier RNA Stock Solution (1 µg/µl, please refer to page 2 for preparation of Carrier RNA Stock Solution). Close the lid, and mix by inverting. Incubate at 70°C for 10 min. Vortex the tube for 10 sec every 3 min to improve lysis. Centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.

   Note: A white precipitate may form when Buffer GB is added. The precipitate does not interfere with the procedure and will dissolve during the heat incubation at 70°C. If the precipitate will not dissolve, it indicates that the cell is not completely lysed and may results in low yield of DNA and impurity in DNA.
5. Add 200 µl ethanol (96-100%) (If room temperature exceeds 25°C, cool the ethanol on ice before adding to the 1.5 ml microcentrifuge tube), close the lid, and mix by inverting the tube gently. Incubate for 5 min at room temperature (15-30°C). Briefly centrifuge to remove drops from inside the lid.
6. Carefully transfer the entire lysate from step 5 to the Spin Column CR2 (in a 2 ml Collection Tube) without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and replace the Spin Column CR2 in the Collection Tube.
7. Carefully open the Spin Column CR2 and add 500 µl Buffer GD **(Ensure ethanol has been added before use)** without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through. Replace the Spin Column CR2 in the Collection Tube.

8. Carefully open the Spin Column CR2 and add 600 µl Buffer PW **(Ensure ethanol has been added before use)** without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and replace the Spin Column CR2 in the Collection Tube.
9. Repeat step 8.
10. Replace the Spin Column CR2 in the Collection Tube, and centrifuge at 12,000 rpm (~13,400 × g) for 2 min. Discard the flow-through and incubate Spin Column CR2 in room temperature for 2-5 min to dry the membrane completely.

    Note: This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.
11. Place the Spin Column CR2 in a clean 1.5 ml microcentrifuge tube (not provided). Apply 20-50 µl Buffer TB to the center of the membrane. Close the lid and incubate at room temperature for 2-5 min. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min.

    Note: The elution volume should not be less than 20 µl since smaller volume will affect recovery efficiency. For high yield of DNA, the flow-through containing DNA can be added to CR2 again. Incubate the column at room temperature for 2 min. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min. The pH value of eluted buffer has some influence in eluting; Buffer TB or distilled water (pH 7.0-8.5) is suggested to elute DNA. For long-term storage of DNA, eluting in Buffer TB and store at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.

Protocol:

<3> Isolation of Circulating Nucleic Acid/cell free Nucleic Acid from Plasma/Serum

Ensure that ethanol (96-100%) has been added to Buffer GD and Buffer PW according to the instructions at the first use.

1. Pipet 100-200 µl serum/plasma into a 2 ml microcentrifuge tube (not supplied). If the sample is less than 100 µl, add Buffer GA to a final volume of 100 µl.
2. Add 20 µl Proteinase K, vortex thoroughly to mix.
3. Add 200 µl Buffer GB (If the initial volume of serum/plasma is lower than 50 µl, add 1 µl Carrier RNA Stock Solution (1 µg/µl, please refer to page 2 for preparation of Carrier RNA Stock Solution) to Buffer GB). Close the lid, and mix by inverting gently. Incubate at 56°C for 10 min, and shake the 2 ml microcentrifuge tube during that time gently. Briefly centrifuge the 2 ml microcentrifuge tube to remove drops from inside the lid.

   Note: A white precipitate may form when Buffer GB is added. The precipitate does not interfere with the procedure and will dissolve during the heat incubation at 56°C. If the precipitate will not dissolve, it indicates that the cell is not completely lysed and may result in low yield of DNA and impurity in DNA.
4. Add 200 µl ethanol (96-100%) (If room temperature exceeds 25°C, cool the ethanol on ice before adding to the 2 ml microcentrifuge tube), close the lid, and mix thoroughly by inverting gently. Incubate for 5 min at room temperature (15-30°C). Briefly centrifuge the 2 ml microtube to remove drops from inside the lid.
5. Carefully transfer the entire lysate from step 4 to the Spin Column CR2 (in a 2 ml Collection Tube) without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through. Replace the Spin Column CR2 in the collection tube
6. Carefully open the Spin Column CR2 and add 500 µl Buffer GD **(Ensure ethanol has been added before use)** without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and replace the Spin Column CR2 in the Collection Tube.

7. Carefully open the Spin Column CR2 and add 600 µl Buffer PW **(Ensure ethanol has been added before use)** without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and replace the Spin Column CR2 in the Collection Tube.
8. Repeat step 7.
9. Replace the Spin Column CR2 in the Collection Tube, centrifuge at 12,000 rpm (~13,400 × g) for 2 min and discard the flow-through. Incubate Spin Column CR2 in room temperature for 2-5 min to dry the membrane completely.

   Note: This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.
10. Place the Spin Column CR2 in a clean 1.5 ml microcentrifuge tube (not provided). Apply 20-50 µl Buffer TB to the center of the membrane. Close the lid and incubate at room temperature for 2-5 min. Centrifuge at 12,000 rpm (~134, 00 × g) for 2 min.

    Note: The elution volume should not be less than 20 µl since smaller volume will affect recovery efficiency. For high yield of DNA, the flow-through containing DNA can be added to CR2 again, incubation at room temperature for 2 min. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min. The pH value of eluted buffer has some influence in eluting; Buffer TB or distilled water (pH 7.0-8.5) is suggested to elute DNA. For long-term storage of DNA, eluting in Buffer TB and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.

Protocol:

<4> Isolation of genomic DNA from mouthwash

Ensure that ethanol (96-100%) has been added to Buffer GD and Buffer PW according to the instructions at the first use.

1. Add 10-20 ml mouthwash into 50 ml sterilized tube (not supplied). Centrifuge at 800 rpm (1,800 × g) for 5 min. Remove the supernatant carefully.
2. Add 200 µl Buffer GA to resuspend the precipitate and transfer all suspension solution into a 1.5 ml microcentrifuge tube.
3. Add 20 µl Proteinase K and vortex for 10 sec to mix. Incubate at 56°C for 60 min, mix by vortex every 15 min.
4. Add 200 µl Buffer GB and 1 µl Carrier RNA Stock Solution (1 µg/µl, please refer to page 2 for preparation of Carrier RNA Stock Solution). Close the lid, and mix by inverting thoroughly. Incubate at 70°C for 10 min. Vortex for 10 sec every 3 min and briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.

   Note: A white precipitate may form when Buffer GB is added. The precipitate does not interfere with the procedure and will dissolve during the heat incubation at 70°C. If the precipitate will not dissolve, it indicates that the cell is not completely lysed and may results in low yield of DNA and impurity in DNA.
5. Add 200 µl ethanol (96-100%), close the lid, and mix thoroughly by inverting the tube. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.

   Note: A white precipitate may form when ethanol is added but this does not affect DNA yield.
6. Carefully transfer the entire lysate from step 5 to the Spin Column CR2 (in a 2 ml Collection Tube) without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through. Replace the Spin Column CR2 in the collection tube.
7. Carefully open the Spin Column CR2 and add 500 µl Buffer GD **(Ensure ethanol has been added before use)** without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and replace the Spin Column CR2 in the Collection Tube.

8. Carefully open the Spin Column CR2 and add 600 µl Buffer PW **(Ensure ethanol has been added before use)** without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through.
9. Repeat step 8.
10. Replace the Spin Column CR2 in the Collection Tube, and centrifuge at 12,000 rpm (~13,400 × g) for 2 min. Discard the flow-through and place Spin Column CR2 in room temperature (15-30°C) for 2-5 min to dry the membrane completely.

    Note: This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.
11. Place the Spin Column CR2 in a clean 1.5 ml microcentrifuge tube (not provided). Apply 20-50 µl Buffer TB to the center of the membrane. Close the lid and incubate at room temperature for 2-5 min. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min.

    Note: The elution volume should not be less than 20 µl since smaller volume will affect recovery efficiency. For high yield of DNA, the flow-through containing DNA can be added to CR2 again, incubation at room temperature for 2 min. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min. The pH value of eluted buffer has some influence on eluting; Buffer TB or distilled water (pH 7.0-8.5) is suggested to elute DNA. For long-term storage of DNA, eluting in Buffer TB and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.

Protocol:

<5> Isolation of Genomic DNA from hair follicles

Ensure that ethanol (96-100%) has been added to Buffer GD and Buffer PW according to the instructions at the first use.

Please prepare 1 M DTT solution before use.

1. Material treatment: hair with hair follicle

   Pipet 250 µl GA, 20 µl Proteinase K and 20 µl 1 M DTT into a 1.5 ml microcentrifuge tube (not supplied), add 1 cm length of hair follicles from the bottom of hair into the 1.5 ml microcentrifuge tube. Mix by vortex for 10 sec.

2. Incubate at 56°C at least 60 min until the sample has been digested thoroughly and vortex for 10 sec every 20 min or incubate in water-bath shaker to digest. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.

   Note: lysis time varies depending on sample types from one hour to overnight. And overnight digestion does not influence the results. Feather stem cannot be dissolved thoroughly even after overnight digestion, so for residual feather stem sample, directly centrifuge and pipet supernatant for downstream applications.

3. Add 300 µl Buffer GB and 1 µl Carrier RNA Stock Solution (1 µg/µl, please refer to page 2 for preparation of Carrier RNA Stock Solution). Close the lid, and mix by inverting thoroughly.

4. Incubate at 56°C for 10 min and vortex for 10 sec every 3 min.

5. Add 300 µl ethanol (96-100%), close the lid, and mix thoroughly by pulse-vortex for 15 sec. Centrifuge the 1.5 ml microcentrifuge tube briefly to remove drops from inside the lid.

6. Carefully transfer the entire lysate from step 5 to the Spin Column CR2 (in a 2 ml Collection Tube) in two times without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and replace the Spin Column CR2 in the collection tube.

7. Carefully open the Spin Column CR2 and add 500 µl Buffer GD **(Ensure ethanol has been added before use)** without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and replace the Spin Column CR2 in the Collection Tube.

8. Carefully open the Spin Column CR2 and add 600 µl Buffer PW **(Ensure ethanol has been added before use)** without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and replace the Spin Column CR2 in the Collection Tube.
9. Repeat step 8.
10. Replace the Spin Column CR2 in the Collection Tube, centrifuge at 12,000 rpm (~13,400 × g) for 2 min and discard the flow-through. Incubate Spin Column CR2 in room temperature (15-30°C) for several minutes to dry the membrane completely.

    Note: This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.
11. Place the Spin Column CR2 in a clean 1.5 ml microcentrifuge tube (not provided) and apply 20-50 µl Buffer TB to the center of the membrane. Close the lid and incubate at room temperature for 2-5 min. Centrifuge at 12,000 rpm (~134, 00 × g) for 2 min.

    Note: The elution volume should not be less than 20 µl since smaller volume will affect recovery efficiency. For high yield of DNA, the flow-through containing DNA can be added to CR2 again, Incubation at room temperature for 2 min. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min. The pH value of eluted buffer has some influence in eluting; Buffer TB or distilled water (pH 7.0-8.5) is suggested to elute DNA. For long-term storage of DNA, eluting in Buffer TB and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.

Protocol:

<6> Isolation of Genomic DNA from tiny amount of tissues

Ensure that ethanol (96-100%) has been added to Buffer GD and Buffer PW according to the instructions at the first use.

1. Add tissue (<10 mg) into a 1.5 ml microcentrifuge tube (not supplied). Immediately add 180 µl Buffer GA and equilibrate to room temperature (15-30°C).
2. Add 20 µl Proteinase K and mix by vortex for 10 sec.
3. Incubate at 56°C for at least 30-60 min until the sample has been digested thoroughly. During the period vortex every 15 min or incubate in water-bath shaker to digest. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.
4. Add 200 µl Buffer GB and 1 µl Carrier RNA Stock Solution (1 µg/µl, please refer to page 2 for preparation of Carrier RNA Stock Solution). Close the lid, and mix by inverting thoroughly and incubate at 70°C for 10 min. Mix by pulse-vortex for 10 sec every 3 min and briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.
5. Add 200 µl ethanol (96-100%) (If room temperature exceeds 25°C, cool the ethanol on ice before adding to the 1.5 ml microcentrifuge tube). Close the lid, and mix thoroughly by inverting the tubes. Incubate for 5 min at room temperature. Briefly centrifuge the 1.5 ml microcentrifuge tube to remove drops from inside the lid.
6. Carefully transfer the entire lysate from step 5 to the Spin Column CR2 (in a 2 ml Collection Tube) without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through. Replace the Spin Column CR2 in the collection tube.
7. Carefully open the Spin Column CR2 and add 500 µl Buffer GD (Ensure ethanol has been added before use) without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and replace the Spin Column CR2 in the Collection Tube.

8. Carefully open the Spin Column CR2 and add 600 µl Buffer PW (Ensure ethanol has been added before use) without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through and replace the Spin Column CR2 in the Collection Tube.
9. Repeat step 8.
10. Replace the Spin Column CR2 in the collection tube, centrifuge at 12,000 rpm (~13,400 × g) for 2 min and discard the flow-through. Incubate Spin Column CR2 in room temperature for several minutes to dry the membrane completely.

    Note: This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.
11. Place the Spin Column CR2 in a clean 1.5 ml microcentrifuge tube (not provided) and apply 20-50 µl Buffer TB to the center of the membrane. Close the lid and incubate at room temperature for 2-5 min. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min.

    Note: The elution volume should not be less than 20 µl since smaller volume will affect recovery efficiency. For high yield of DNA, the flow-through containing DNA can be added to CR2 again, Incubation at room temperature for 2 min. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min. The pH value of eluted buffer has some influence in eluting; Buffer TB or distilled water (pH 7.0-8.5) is suggested to elute DNA. For long-term storage of DNA, eluting in Buffer TB and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.

Protocol:

<7> Isolation of Genomic DNA from Microdissected Tissues including Formalin Fixed Microdissected Tissues

Ensure that ethanol (96–100%) has been added to Buffer GD and Buffer PW according to the instructions at the first use.

1. Pipet 15 µl Buffer GA into a 0.2 ml microcentrifuge tube, then add the microdissected tissues into 0.2 ml microcentrifuge tube.
2. Add 10 µl Proteinase K and mix by vortex for 10 sec.
3. Incubate at 56°C for 3 h (For formalin fixed microdissected tissues, incubate at 56°C for 16 h) with occasional vortex until the sample has been digested or incubate in water-bath shaker to digest. Briefly centrifuge the 0.2 ml microcentrifuge tube to remove drops from inside the lid.
4. Add 25 µl Buffer GA, mix by vortex and add 50 µl Buffer GB and 1 µl Carrier RNA Stock Solution (1 µg/µl, please refer to page 2 for preparation of Carrier RNA Stock Solution). Close the lid, and mix by pulse-vortex for 10 sec. Briefly centrifuge the 0.2 ml microcentrifuge tube to remove drops from inside the lid.
5. Add 50 µl ethanol (96-100%) (If room temperature exceeds 25°C, cool the ethanol on ice before adding to the microcentrifuge tube). Close the lid, and mix thoroughly by inverting. Incubate for 5 min at room temperature (15-30°C). Briefly centrifuge the 0.2 ml microcentrifuge tube to remove drops from inside the lid.
6. Carefully transfer the entire lysate from step 5 to the Spin Column CR2 (in a 2 ml Collection Tube) without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through. Replace the Spin Column CR2 in the Collection Tube.
7. Carefully open the Spin Column CR2 and add 500 µl Buffer GD (Ensure ethanol has been added before use) without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through. Replace the Spin Column CR2 in the Collection Tube.

8. Carefully open the Spin Column CR2 and add 600 µl Buffer PW (Ensure ethanol has been added before use) without wetting the rim. Close the lid, and centrifuge at 12,000 rpm (~13,400 × g) for 30 sec. Discard the flow-through. Replace the Spin Column CR2 in the Collection Tube,
9. Repeat step 8.
10. Replace the Spin Column CR2 in the Collection Tube, centrifuge at 12,000 rpm (~13,400 × g) for 2 min and discard the flow-through. Incubate Spin Column CR2 in room temperature (15-30°C) for 2-5 min to dry the membrane completely.

    Note: This step is necessary, since ethanol carryover into the eluate may interfere with some downstream applications.
11. Place the Spin Column CR2 in a clean 1.5 ml microtube (not provided) and apply 20-50 µl Buffer TB to the center of the membrane. Close the lid and incubate at room temperature for 2-5 min. Centrifuge at 12,000 rpm (13,400 × g) for 2 min.

    Note: The elution volume should not be less than 20 µl since smaller volume will affect recovery efficiency. For high yield of DNA, the flow-through containing DNA can be added to CR2 again. Incubate the column at room temperature for 2 min. Centrifuge at 12,000 rpm (~13,400 × g) for 2 min. The pH value of eluted buffer has some influence in eluting; Buffer TB or distilled water (pH 7.0-8.5) is suggested to elute DNA. For long-term storage of DNA, eluting in Buffer TB and storing at -20°C is recommended, since DNA stored in water is subject to acid hydrolysis.