

Quick-Start Manual

Revision 1.0 Dec 2023

Notes before starting:

- Add 5 mL Protease Dissolve Buffer to the Proteinase K, and store at -20~8°C after dissolve.
- Add 2.5 mL Protease Dissolve Buffer to the RNase A, and store at -20~8°C after dissolve.
- Dilute Buffer BW1 with 140 mL 100% ethanol and store at room temperature.
- Dilute Buffer BD with 80 mL 100% ethanol and store at room temperature
- Prepare 75% Ethanol using Absolute Ethanol and store at room temperature.

Manual Extraction

Sample Preparation

A. Solid tissue (1-20 mg)

1. Cut ~20 mg tissue into small pieces and transfer into a new 1.5 mL microcentrifuge tube. Add 200 μ L Buffer ATL and 20 μ L Proteinase K and incubate with shaker at 55°C for 30 to 180 mins.
2. (Optional) Add 10 μ L RNase A into the lysate and incubate at room temperature for 10 mins.
3. Add 200 μ L Buffer AL into the samples, mix thoroughly by vortexing to yield homogenous solution.
4. Incubate at 70°C for 10 mins.

B. Anticoagulated blood or Plasma (200 μ L)

1. Transfer 20 μ L Proteinase K to a new 1.5 mL microcentrifuge tube.
2. Add 200 μ L whole blood, plasma, or other body fluids into the tube. Shake to mix for 5 seconds.
3. Add 200 μ L Buffer AL into the samples. Invert the tube for 3 - 5 times, and vortex at maximum speed for 10 seconds. Incubate at 70°C for 10 mins.

C. Saliva sample

1. Add 20 μ L Proteinase K and 10 μ L RNase A into a 1.5 mL microcentrifuge tube.
2. Transfer 450 μ L saliva to the tube and shake to mix for 5 seconds.
3. Incubate at 55°C for 30 mins.

D. Culture cells

1. Collect cells (<2 x10⁶) by centrifuging at 2,000 x g for 5 mins. Remove the supernatant.
2. Add 200 μ L Buffer PBS, 20 μ L Proteinase K, and 10 μ L RNase A into the sample. Resuspend the cells by vortexing.
3. Add 200 μ L Buffer AL and vortex for 10 seconds. Incubate the mixture at 70°C for 10 mins.

E. Semen sample

1. Transfer 100 μ L semen to a 1.5 mL microcentrifuge tube.
2. Add 100 μ L Buffer ATL, 10 μ L DTT Solution (1M), and 20 μ L Proteinase K into the samples. Shake at 55°C for 30 mins.
3. Add 200 μ L Buffer AL into the sample, then vortex to mix and incubate at 70°C for 10 mins.

F. Swab DNA extraction

1. Transfer the swabs to a 2.0 mL microcentrifuge tube.
2. Add 500 μ L ATL and 20 μ L Proteinase K to the swab. Shake at 55°C for 15 to 30 mins.
3. Transfer the supernatant into a new 2.0 mL microcentrifuge tube.

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Sample Preparation

G. Blood stains/Seminal stains

1. Transfer 3 slices (3mm) to the 2.0 mL microcentrifuge tube.
2. Add 250 μ L Buffer ATL and 20 μ L Proteinase K to the sample. Shake at high speed for 30 to 60 mins at 55°C.
3. Add 250 μ L Buffer AL into the samples, Shake at high speed for 10 mins at 70°C.
4. Centrifuge at 13,000 x g for 1 min.
5. Transfer 400 μ L of the supernatant to a new 2.0 mL microcentrifuge tube.

H. FFPE Samples

1. Using a scalpel, trim excess paraffin off the sample block. Transfer 1 to 3 sections (5 - 10 μ m) into a 1.5 mL microcentrifuge tube.
2. Remove Paraffin by xylene or Buffer DPS (not provided).
3. Add 200 μ L Buffer ATL and 20 μ L Proteinase K into the sample, mix well and incubate at 56°C for 60 mins, and 90°C for 60 mins.
4. Cool the sample to room temperature and add 200 μ L Buffer AL. Mix well before proceeding to extraction step.

Extraction

1. Add 20 μ L Magbeads Particles and 400 μ L Buffer BD to the samples or the supernatant. Mix thoroughly by inverting for 15~30 times. Incubate for 3 min with occasional inverting to mix. Place the tube on the magnetic stand for 1 min until the beads have formed a tight pellet. Then remove the supernatant.
2. Add 600 μ L Buffer BW1 and vortex for 15 sec to resuspend the beads. Place the tube on the magnetic stand for 1 min until the beads have formed a tight pellet. Then remove the supernatant.
3. Add 600 μ L Buffer BW1 and vortex for 15 sec to resuspend the beads. Place the tube on the magnetic stand for 1 min until the beads have form a tight pellet. Then remove the supernatant.
4. Add 600 μ L 75% ethanol, and vortex for 15 sec to resuspend the beads. Place the tube on the magnetic stand for 1 min until the beads have formed a tight pellet. Then remove the supernatant.
5. Add 600 μ L 75% ethanol, and vortex for 15 sec to resuspend beads. Place the tube on the magnetic stand for 1 min until the beads have formed a tight pellet. Then remove the supernatant.
6. Centrifuge briefly to collect the liquid on the tube. Place the tube on the magnetic stand and remove all the liquid carefully. Air dry for 10 minutes.
7. Add 50~100 μ L Elution Buffer to the sample, re-suspend the beads by vortex. Incubate at 55°C for 10 minutes with shaking. If there is no shaking device, vortex 2~3 times to mix.
8. Place the tube on the magnetic stand for 2 mins. Transfer the supernatant containing the purified DNA to a clean 1.5 mL tube.

