Recommended Starting Material

The size of recommended starting material to use with determined lysis volume are listed here.

Table: Recommended starting material and Lysis Buffer amount

Sample	Size of Starting Material	Lysis Buffer Amount
Under 18 weeks	5-6 ml of amniotic fluid	250 µl
Above 18 weeks	2-3 ml of amniotic fluid	

Protocol

Isolation of Genomic DNA (based on silica technology)

Sample type:

- Amniotic fluid

Some tips to know:

- All centrifugation steps are carried out at room temperature (15–25°C).
- If RNase treatment is desired, Prime-RNase can be ordered separately from ROJETechnologies, Cat NO. EB983013.
- Phosphate buffered saline (PBS) can be prepared as written in appendix 4 in DNJia AmnioPure Kit or can be ordered separately from ROJETechnologies, Cat NO. BU983026.
- Do not forget to add the appropriate amount of Ethanol (96–100%) to BW1 and BW2 buffers (BWB1 and BWB2) as indicated on the bottle, before using for the first time.
- If AL Buffer (ALB) forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Process

1. Centrifuge 1.5 ml of the amniotic fluid for 10 min at 5000 RPM in a clean microcentrifuge tube, discard the supernatant and repeat this step until you get pellet from whole sample mentioned in the table (based on the gestation week).
2. Add 20 µl RJ-Protease to the pellet. Add 250 µl PBS to cell pellets to the tube. Then add 250 µl ALB. Pulse vortex for 15 s and incubate at 56 °C for 25 min.

   Optional: Pulse-vortexing for 3 times during incubation may increase the DNA yield.

   Optional: This protocol is specialized for DNA isolation. For usual and routine applications RNase treatment isn't needed. If RNA-free genomic DNA is required, add 10 µl Prime-RNase A (Cat NO. EB983013), mix by vortexing, and incubate for 5 min at room temperature before going to step 2.

3. Add 250 µl Ethanol (96-100%, molecular biology grade) to the lysate, mix by pulse vortexing for 15 s, then centrifuge briefly.
4. Gently, pipette the mixture to a spin column placed in a 2 ml collection tube (supplied in the kit box). Centrifuge at 8000 rpm for 1 min. Discard flow-through.

Note: If the lysate did not pass the column, centrifuge at full speed until it passes through the column.

5. Add 600 µl BWB1, incubate for 2 min at room temperature (15-25℃) and centrifuge for 1 min at 8000 rpm. Then discard the flow-through.
6. Add 600 µl BWB2, incubate for 2 min at room temperature (15-25℃) and centrifuge for 3 min at 14000 rpm. Discard the flow-through and centrifuge for 1 min at 14000. Then discard both the flow-through and the collection tube. Place the spin column in a new clean 1.5 ml microcentrifuge tube (not provided).
7. Pipette 25-30 µl RRB directly onto spin column. Incubate at 37℃ for 1-5 min. Centrifuge it at 8000 rpm for 1 min.

   Note: If the expected DNA yield is more than the yield from pervious step, put the spin column on a new microtube and add another 25-30 µl RRB, incubate for 5 min at 37℃. Then, centrifuge for 1 min at 8000 rpm.