

Spical Protocol for Extraction of DNA from Amniotic Fluid:

1. Transfer up to 1 ~ 3 ml amniotic fluid to a centrifuge tube (not provided). Centrifuge at 10,000 x g for 5 min then remove the supernatant.
2. Wash the cell pellet with 1 ml of PBS. Centrifuge at 10,000 x g for 3 min then remove the supernatant completely.
3. Add 200 µl of PBS and resuspend the cells by pipetting. Transfer the sample mixture to a 1.5 ml microcentrifuge tube. (not provided)
4. **(Optional):** If RNA-free genomic DNA is required, add 4 µl of 100 mg/ml RNase A to the sample and incubate for 2 min at room temperature.
5. Add 20 µl Proteinase K and 200 µl FABG Buffer to the sample. **Mix thoroughly by pulse-vortexing.**
- Do not add Proteinase K directly to FABG Buffer.
6. Incubate at 60 °C for 15 minutes to lyse the sample. **During incubation, vortex the sample every 3-5 minutes.**
7. Centrifuge the tube at 10,000 x g for 3 min and transfer the clarified supernatant to a new 1.5 ml microcentrifuge tube. (not provided)
8. Add 200 µl of ethanol (96- 100 %) to the sample mixture. **Mix thoroughly by pulse-vortexing for 30 sec.**
9. Briefly spin the tube to remove drops from the inside of the lid.
10. Place a FABG Mini Column in a Collection Tube. Transfer the sample mixture (including any precipitate) carefully to the FABG Mini Column. Centrifuge at 8,000 x g for 30 sec **then place the FABG Mini Column to a new Collection Tube.**
11. Wash the FABG Mini Column with 500 µl W1 Buffer by centrifuge at 8,000 x g for 30 sec then discard the flow-through.
12. Wash the FABG Mini Column with 750 µl Wash Buffer by centrifuge at 8,000 x g for 30 sec then discard the flow-through.
- Make sure that ethanol has been added into Wash Buffer when first open.
13. **Centrifuge the FABG Mini Column at full speed (~18,000 x g) for an additional 3 minutes to dry the column.**
14. Place the FABG Mini Column to Elution Tube.
15. Add 100 ~ 200 µl of Elution Buffer or ddH₂O (pH 7.5- 9.0) to the membrane center of FABG Mini Column. **Stand FABG Column for 3 minutes.**
- **Important Step!** For effective elution, make sure that the elution solution is dispensed onto the membrane center and is absorbed completely.
16. Centrifuge at full speed (~18,000 x g) for 1 min to elute total DNA