Genomic DNA isolation procedure for amniotic fluid samples

1. Take at least 2 ml of amniotic fluid fresh or 50000-150000 cells of cultured amniotic fluid, previously re-suspended, and put it into a provided 2 ml tube.

   Note: *Yield depends on gestation week of the fetal material from which the sample was sourced. The kit has been tested to yield >1µgr using starting materials of approximately 2 ml of amniotic fluid obtained from fetal amniocentesis at 15 weeks of gestation or more. However, using larger volume when possible is recommended.*

2. Centrifuge at 11200 g (approximately 12000 rpm) for 5 minutes at room temperature.
3. Discard the supernatant with care.

   Note: *Repeat steps 1 to 3 if sample volume is larger than 2 ml, until all sample has been centrifuged.*

4. Add 300 µl of **Solution A** and vortex gently.

   Note: *Mix gently before addition if Solution A was stored at 4°C*

5. Incubate at 55°C for 30 minutes. Use of a horizontal shaker (at 100-150 rpm) is optional but preferable.

   Optional RNase treatment: *heat sample at 70°C for 5 minutes. Add RNase (not provided) 100 µg/ml (final concentration) and incubate at 37°C for 5 minutes. Finally, continue to step 6.*

6. Cool the samples (from step 5) by incubating approximately 5 minutes on ice or refrigerator.
7. Add 100 µl of **Solution B** and vortex gently.
8. Centrifuge at 17000 g (approx. 13500 rpm) for 10 min., at room temperature
9. Carefully pipet the supernatant into a provided 1.5 ml tube, discarding the remaining pellet.
10. Add 40 µl of **Solution C** and 400 µl of **Solution D**.
11. Shake slightly by inverting the tube several times until a homogenous solution is observed.
12. Incubate the tube at room temperature for 10 minutes in a vertical position.
13. Centrifuge at 11200 g (12000rpm) for 5min at room temperature.

    Note: *Usually, a little pellet forms.*

14. Discard the supernatant with care.
15. Add 500 µl of **Solution E**.
16. Centrifuge at 11200 g (12000 rpm) for 5 minutes at room temperature.

    Optional: *An additional wash with 70% ethanol can be done to avoid salt excess in the final sample.*

17. Discard the supernatant with care (*) and place the tube (containing the pellet) open for 5-10 minutes to dry the pellet.

    (*)Pellet is easily removable from the bottom of the tube, so supernatant must be discarded very carefully to avoid sample loss.

18. Add 30-50 µl of Solution F and pipet up and down carefully to resuspend the genomic DNA.

    Note: Nuclease-free water can be used but is not recommended for long time storage.

19. Optional, incubate the tube at 37°C for 30 minutes to help DNA solubilisation.
20. Use immediately or store at 4°C (if used during the next 48 hours) or at -20°C (for longer storage).

High DNA quality, maximun DNA quantity extracted from amniotic fluid and chorionic villus samples. Suitable for array-CGH/SNP.

Genomic DNA isolation procedure for chorionic villus samples

1. Take a small amount of chorionic villi (1-5 mg) or 50000-150000 cells of cultured chorionic villi and place it into a provided 2 ml tube.
2. Add 300 µl of **Solution A** and vortex gently. If you start with cell culture first centrifuge at 11200 g (approximately 12000 rpm) for 5min at room temperature and discard the supernatant with care. Then add 300 µl of **Solution A**.

   Note: *Mix gently before addition if Solution A was stored at 4ºC.*
3. Incubate at 55ºC for 60 minutes. Use of a horizontal shaker (at 100- 150 rpm) is optional but preferable

   Optional RNase treatment: *heat sample at 70ºC for 5 minutes. Add RNase (not provided) 100 µg/ml (final concentration) and incubate at 37ºC for 5 minutes. Finally, continue to step4.*
4. *Cool the samples (from step 3) by incubating approximately 5 minutes on ice or refrigerator.*
5. Add 100 µl of **Solution B** and vortex gently.
6. Centrifuge at 17000 g (approx. 13500 rpm) for 10 min., at room temperature.
7. Carefully pipet the supernatant into a provided 1.5 ml tube, discarding the remaining pellet.
8. Add 40 µl of **Solution C** and 400 µl of **Solution D**.
9. Shake slightly by inverting the tube several times until a homogenous solution is observed.
10. Incubate the tube at room temperature for 10min in a vertical position.
11. Centrifuge at 11200 g (12000 rpm) for 5 minutes, at room temperature.

    Note: *Usually a little pellet forms.*
12. Discard the supernatant with care.
13. Add 500 µl of **Solution E**.
14. Centrifuge at 11200 g (12000 rpm) for 5 minutes at room temperature.

    Optional: *An additional wash with 70% ethanol can be done to avoid salt excess in the final sample.*
15. Discard the supernatant with care(*) and place the tube (containing the pellet) open for 5-10 minutes to dry the pellet.

    (*)Pellet is easily removable from the bottom of the tube, so supernatant must be discarded very carefully to avoid sample loss.
16. Add 30-50 µl of **Solution F** and pipet up and down carefully to resuspend the genomic DNA.

    Note: *Nuclease-free water can be used but is not recommended for long time storage.*
17. Optional, incubate the tube at 37ºC for 30 minutes to help DNA solubilisation.
18. Use immediately or store at 4ºC (if used during the next 48 hours) or at -20ºC (for longer storage).

High DNA quality, maximun DNA quantity extracted from amniotic fluid and chorionic villus samples. Suitable for array-CGH/SNP.