

AMNOKIA

Amniotic Fluid DNA Isolation Kit

Cat. No:

FPKT002.0025

FPKT002.0050

FPKT002.0100

Contents:

Components	25 preps	50 preps	100 preps
AM1 Buffer (RED)	50 ml	100 ml	200 ml
AM2 Buffer (Orange)	7.5 ml	15 ml	30 ml
AM3 Buffer (Yellow)	7.5 ml	15 ml	30 ml
AM4 Buffer (Green)	5 ml	10 ml	20 ml
AM5 Buffer (Blue)	1 ml	2 ml	4 ml
EB Buffer	1.5 ml	2.5 ml	5 ml
Column and collection tubes	25 each	50 each	100 each

Kit storage:

⚠ This kit should be stored at room temperature. If properly stored, all kit components are stable until the expiration date printed on the label.

⚠ After adding **absolute ethanol** to **AM5 Buffer**, Store the **AM5 Buffer** at +2 to +8°C

Additional Equipment and Reagent required

- Absolute ethanol
- Chloroform
- Standard tabletop microcentrifuge capable of 13,000 x g centrifugal force
- Microcentrifuge tubes, 1.5 ml, sterile

Application

This kit provides a convenient and rapid method to isolate total DNA from fresh, frozen and preserved Amniotic Fluid Samples. The purified DNA is of the highest quality and is fully compatible with all downstream applications such as PCR, qPCR, NGS and microarrays since all humic acid substances and other PCR inhibitors are removed during the isolation process. The procedure is optimized to achieve reliable results within **12 min**.

Handling Requirements and Safety Information

⚠ All solutions are clear and should not be used when precipitates have formed. Warm the solutions at +15 to +25°C or in a 37°C water bath until the precipitates have dissolved.

⚠ AM4 Buffer contain guanidinium hydrochloride which is an irritant.

⚠ Do not allow AM4 Buffer to touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water.

⚠ If you spill the reagent, dilute the spill with water before wiping it up.

⚠ Do not use any modified ethanol.

⚠ Do not pool reagents from different lot numbers.

⚠ Immediately after usage, close all bottles in order to avoid leakage, varying buffer concentrations or buffer conditions.

⚠ After first opening store all bottles in an upright position.

⚠ Do not allow the AM4 Buffer to mix with sodium

hypochlorite found in commercial bleach solutions. This mixture can produce a highly toxic gas.

⚠ Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.

⚠ Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease free pipet tips only, to remove aliquots from reagent bottles.

preparation procedure:

Working Solution Preparation

FPKT001.0025-25 preps

⚠ Add 4 ml absolute ethanol to AM5 Buffer Bottle.

FPKT001.0050-50 preps

⚠ Add 8 ml absolute ethanol to AM5 Buffer Bottle.

FPKT001.0100-100 preps

⚠ Add 16 ml absolute ethanol to AM5 Buffer Bottle.

Please note that the Ethanol concentration of a AM5 Buffer may decrease during long term storage resulting in a drop-down of the final DNA yield.

⚠ before starting Incubate the **EB buffer at 55 to 65 ° C** until the end of the protocol to obtain the maximum yields.

Protocol



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1. Add 1 ml AM1 Buffer to the 2ml microtube and **add 800 µl Amniotic Fluid** sample. Mix gently (don't Vortex) and centrifuge at maximum speed for 1 minutes.

2. Discard the supernatant and add 1ml AM1 Buffer again. Mix gently (don't Vortex) and **centrifuge at maximum speed for 1 minutes.**

3. Discard the supernatant and Add 300 µl AM2 Buffer. Pipette completely.

4. Add 300 µl Am3 Buffer then **add 600 µl Chloroform** mix vigorously for 10 seconds (don't Vortex). **Centrifuge at 7000 RPM for 4 min.**

5. Transfer the supernatant into a 1.5 ml microtube gently. Add **200 µl of AM4 Buffer** to the sample. **mix gently** (don't Vortex).

6. Transfer the Step 5 solution into a DNA spin column assembled in a clean collection tube (provided). Centrifuge at maximum speed for 1 minutes.

7. Disconnect the DNA spin column from collection tube and discard the flow through solution. Reconnect the DNA spin column to the collection tube.

8. Add 200 µl AM5 Buffer to DNA spin column and centrifuge at maximum speed for 1 minutes. Discard flow through.

9. Place the DNA spin column into the clean new microtube. Add 50 µl of pre-warmed **EB Buffer** or sterile DDW directly onto column membrane and stand for 3 min. centrifuge at maximum speed for 1 minutes.

10. Check 7-10 µl of extracted DNA by 1% agarose gel electrophoresis. Store DNA at -20°C