Amniotic Fluid DNA Extraction kit MANUAL

GENEDIA™ **life Science Co**.

Product # EK1250R

 # EK12100R

 # EK12200R

Unit 1, Ground Floor, No.14 Building, End of the Taghavi Alley,South Bahar St, Kaveh Blvd,Tehran, Iran.
E-Mail: genedialifescience@gmail.com
Phone: +98 21 26601149 +98 21 26601150 +98 922 652 5584

Table of Contents

E-Mail: genedialifescience@gmail.com
Phone: +98 21 26601149 +98 21 26601150 +98 922 652 5584

Introduction

The **GENEDIA™ DNA Extraction from Amniotic Fluid Kit** (spin column) is a fast, simple, and inexpensive means of preparing genomic DNA from 5 ml samples of amniotic fluid for Quantitative Florescence PRC.

The purified genomic DNA is fully digestible with all restriction enzymes tested, and is completely compatible with downstream applications including QF PCR, real-time PCR, RFLP analysis used for paternity testing and southern blot analysis.

Kit Specifications

Purification is based on spin column chromatography using **GENEDIA**'s proprietary resin as the separation matrix. The DNA is preferentially purified from other cellular components without the use of phenol or chloroform. First, samples are digested with the provided Proteinase K and Digestion Buffer –AMFL- using an incubation time which is specific for the recovery of DNA. Next, Absolute Ethanol is added to the lysate, and the solution is loaded onto a spin-column. GENEDIA's column binds nucleic acids in a manner that depends on ionic concentrations, thus only the DNA will bind to the column while the contaminants will be removed in the flowthrough or retained on the top of the resin. The bound DNA is then washed with the provided Wash Solution in order to remove any impurities, and the purified DNA is eluted with AmFE.

Kit Components

Components	Product # EK1250R (50 preps)	Product # EK12100R (100 preps)	Product # EK12200R (200 preps)
AmFL (Lysis)	10ml	20ml	40ml
AmFWB1 (Wash 1)	12 ml	22 ml	43 ml
AmFWB2 (Wash 2)	7 ml	13 ml	25 ml
AmFE (Elution)	2.5 ml	5 ml	10 ml
Proteinase K	1 ml	2 ml	2 ml*2
Spin column	50	100	200
Product insert	1	1	1

Storage Conditions

All components of the **GENEDIA™ Extraction from Amniotic Fluid Kit** should be stored at room temperature (20-25 °C) and are stable for 1 year. To prolong the lifetime of Proteinase K, storage at 2–8°C is recommended.

Recommended Equipment and Reagents

- 56-65°C incubator
- Sampler in 100 to 1000 Microliter size
- Sampler tips
- Benchtop microcentrifuge

E-Mail: genedialifescience@gmail.com
Phone: +98 21 26601149 +98 21 26601150 +98 922 652 5584

- Micropipettors
- 2 ml microcentrifuge tubes
- Vortex
- Absolute Ethanol
- RNase A (if RNA-free DNA is required)

Precautions and Disclaimers

- Prior to using the protocol, Genetic ID recommends that care be taken in homogenizing the sample in a manner that minimizes the risk of inadvertently contaminating the sample. Contamination can occur using improperly cleaned equipment or using poor laboratory practices during homogenization, weighing and labeling of the subsample.
- The Buffer AMFL contains guanidinium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.
- All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5})\,(r)}}$$

Where RCF = required gravitational acceleration (relative centrifugal force in units of g);
r = radius of the rotor in cm; and RPM = the number of revolutions per minute required to achieve the necessary g-force.

- All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.

Notes Prior to Use

• Prepare a working concentration of the **AmFWB1** by adding:

- **5 ml** of 96 - 100% ethanol (not provided) to each of the bottles containing **12 ml** of concentrated **AmFWB1**. This will give a final volume of 17 ml for **Product # EK1250R**
- **9 ml** of 96 - 100% ethanol (not provided) to the supplied bottle containing **22 ml** concentrated **AmFWB1**. This will give a final volume of 31 ml for **Product # EK12100R**
- **18 ml** of 96 - 100% ethanol (not provided) to the supplied bottle containing **43 ml** concentrated **AmFWB1**. This will give a final volume of 61 ml for **Product # EK12200R**

The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.

• Prepare a working concentration of the **AmFWB2** by adding:

- **10 ml** of 96 - 100% ethanol (not provided) to each of the bottles containing **7 ml** of concentrated **AmFWB2**. This will give a final volume of 17 ml for **Product # EK1250R**

E-Mail: genedialifescience@gmail.com
Phone: +98 21 26601149 +98 21 26601150 +98 922 652 5584

- **19 ml** of 96 - 100% ethanol (not provided) to the supplied bottle containing **13 ml** concentrated **AmFWB2**. This will give a final volume of 32 ml for **Product # EK12100R**
- **37 ml** of 96 - 100% ethanol (not provided) to the supplied bottle containing **25 ml** concentrated **AmFWB2**. This will give a final volume of 62 ml for **Product # EK12200R**

The labels on the bottles have a box that may be checked to indicate that the ethanol has been added.

Sampling and Extraction Procedure

A. Sample preparation

1) Transfer **5ml** of Amniotic Fluid to a proper tube (Not provided).
2) Centrifuge at **2,500 x g** (~3,200 RPM) for **3 minutes**.

Note: *If using 2 ml microcentrifuge tube, centrifuge in 3 steps, add the remaining volume to the tube, and repeat the centrifugation.*

3) Discard supernatant through reversal and save the pellet; while removing the supernatant, be careful to not discard the gelatinous appearing pellet.
4) Add **200 µl of AmFL** and **20µl of Proteinase K** solution (20mg/ml) to the sample and vortex for few minutes and incubate at **60℃** for **15 minutes**. After the lysing process wait for the sample to reach room temperature.

(Optional): *RNase A Treatment: If RNA-free genomic DNA is required, add the equivalent of 100 units of RNase A (*not to exceed 10 µL*) to the lysate.*

B. Binding DNA to Column

5) Add **300 µl** Absolute Ethanol (Not provided) and mix by vortexing (2 x 5 s).
6) Assemble a Spin column with collection tube.
7) Transfer onto the column and Incubate for 2 minutes at room temperature.
8) Centrifuge at **8,000 x g** (~ 6,000 RPM) for **2 minutes**.
9) Discard the flowthrough. Reassemble the spin column with its collection tube

Typically, samples will pass through the columns within ≤ 1 minute (in less than 1 minute). If the entire volume has not passed, spin for an additional minute

C. Column Wash

1st wash

10) Add **300 µl Buffer AmFWB1** to the GENEDIA DNA Spin column.
11) Centrifuge at **8,000 x g** (~ 6,000 RPM) for **1 minutes**.
12) Discard the flowthrough. Reassemble the spin column with its collection tube.

2nd wash

13) Add **300 µl Buffer AmFWB2** to the column and centrifuge for **1 minutes** at **8,000 x g** (~ 6,000 RPM).
14) Discard the flowthrough and reassemble the spin column with its collection tube.
15) Spin the column for **2 minutes** in order to thoroughly dry the resin. Discard the collection tube.

E-Mail: genedialifescience@gmail.com
Phone: +98 21 26601149 +98 21 26601150 +98 922 652 5584

D. Elute DNA

16) Place the Spin column in a microcentrifuge tube (provided).
17) Add **50µl AmFE** (preheated to 60°C) to the column.
18) Incubate at room temperature for 2 minutes.
19) Centrifuge at **11,000 x g** (~10,000 rpm) for **1 minutes**.

(Optional): *For an improved yield, elute the sample twice and use after concentration process*

E. Storage of DNA

The purified DNA may be stored at –20°C for a few weeks. It is recommended that samples be placed at –70°C for long term storage.

E-Mail: genedialifescience@gmail.com
Phone: +98 21 26601149 +98 21 26601150 +98 922 652 5584

Troubleshooting Guide

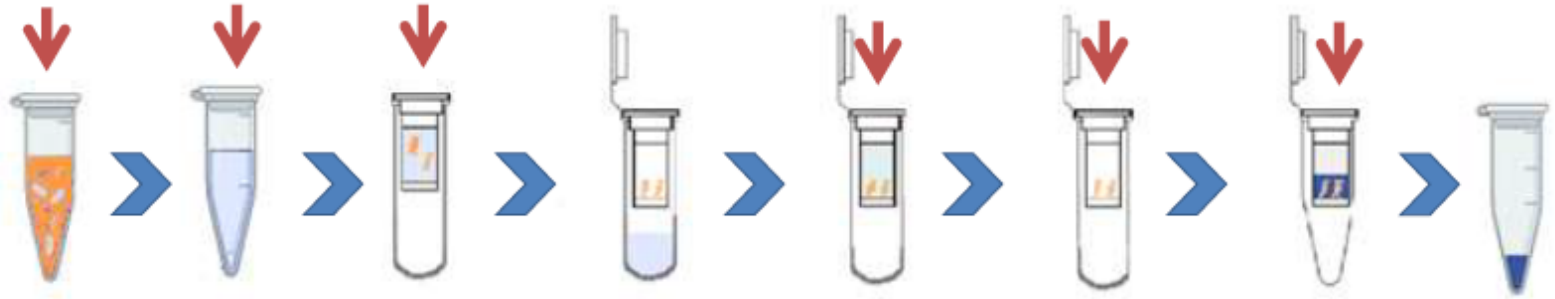
Problem	Possible Cause	Solution and Explanation
No DNA purified	**Reagents added incorrectly**	Add buffers in the correct order so that the sample is bound, washed and eluted in the correct sequence.
	Incomplete elution	Larger elution volumes and longer incubation times can increase yield.
	Reagents added incorrectly	Make sure that buffers have been reconstituted correctly, and that reagents have been added in the correct order.
	Incomplete elution during preparation	Larger elution volumes and longer incubation times can sometimes increase yield. For elution of DNA >10 kb, heat the DNA Elution Buffer to 50°C and extend incubation time to 5 minutes. Multiple rounds of elution can also be performed.
Low DNA performance	**Ethanol has been carried over**	Centrifuge 2nd wash for 1 minute to ensure complete removal.
The spin column is clogged.	**Inefficient cell lysis**	Check Proteinase K activity. Also ensure that correct volume of Lysis Buffer was added to the Amniotic Fluid sample
	The sample is too large	Too many cells were applied to the column. Ensure that Proteinase K and Lysis Buffer are proportionally added as the Amniotic Fluid sample volume is increased. Clogging can be alleviated by centrifuging for a longer period of time until the lysate passes through the column.
Small amounts or no nucleic acids in the eluate	**Proteinase K digestion**	For certain sample types and for DNA isolation use of Proteinase K is required for the sample lysis step. Compare protocols with and without Proteinase K digestion.
	Nucleic acids degraded	Samples should be processed immediately. If necessary, add DNase inhibitor to the sample. Create a nuclease-free environment and ensure that no nucleases are present. Use suitable tips and buffer reservoirs. Check that all buffers have been prepared and stored correctly.

E-Mail: genedialifescience@gmail.com
Phone: +98 21 26601149 +98 21 26601150 +98 922 652 5584

Isolation Method of DNA from Amniotic Fluid

Workflow

Sample Lysis	Nucleic acid Binding	Flow Through	1st Wash	2nd Wash	Elution	
5ml of Amniotic Fluid Centrifuge at 2,500xg 3 min Discard supernatant save the pellet	Add 200 µl of AmFL & 20µl of Proteinase K to cell pellet & vortex & incubate for 15min at 60 °C	Add 300µl Ethanol Transfer onto the column & Incubate for 2 min at RT	Centrifuge at 8,000xg 2 min Discard the flowthrough	Add 300 µl AmFWB1 and Centrifuge at 8,000xg 1 min Discard the flowthrough	Add 300 µl AmFWB2 and Centrifuge at 8,000xg 1 min Discard the flowthrough	Place the column into an Elution tube Add 50 µl AmFE Incubate for 2min at RT Centrifuge at 11,000xg 1 min	Purified DNA



Unit 1, Ground Floor, No.14 Building, End of the Taghavi Alley,South Bahar St, Kaveh Blvd,Tehran, Iran.
E-Mail: genedialifescience@gmail.com
Phone: +98 21 26601149 +98 21 26601150 +98 922 652 5584