Troubleshooting guide

Problem	Possible causes	Suggested solution
Low yield	Starting material is too aged or has been improperly stored	The best yield is typically obtained from fresh samples. DNA yield depends on various factors, including the type, size, age, and storage condition of the starting material. Inappropriate storage, such as blood samples stored at 4°C for more than 5 days, may lead to reduced yields.
	Decreased Proteinase K activity due to improper storage or expiration	Proteinase K must be stored under 4°C after dissolved in PK-Storage buffer for maintenance of proper activity. Proper lysis cannot be performed with degraded Proteinase K. It should be replaced with a new one.
	Inefficient or insufficient lysis	For proper lysis, mix sample and lysis buffer thoroughly.
	Precipitation of Buffer HL	Storage at cool ambient temperature may cause precipitation in Buffer HL. Incubate bottle at 56°C or above until all precipitates are dissolved.
Low concentration of DNA in eluate	Column clogging	Insufficient lysis may lead to column clogging. Mix the sample with each buffer completely. Reduce the starting amount of sample. Extend the Proteinase K incubation time at 56°C.
	Low sample input or a small number of cells in the sample	Either add more starting material or, if needed, minimize the elution volume and re-elute the initial eluate.
Eluate does not perform well in downstream application	Buffer HW1 or HW2 was prepared incorrectly	Check that the Buffer HW1 and HW2 concentrates were diluted with the correct volume of absolute ethanol. Repeat the extraction procedure with new samples, if available.
	Improper storage of DNA	Store isolated DNA at -20°C. Do not store at room temperature.
	Residual ethanol from Buffer HW1 or HW2 that remains in the elute	Care must be taken for eliminating the carryover of Buffer HW1 or HW2 before elution step. The membrane of mini spin column should be kept completely dry using additional centrifugation or air-drying.
	Insufficient lysis	Insufficient lysis causes low DNA purity, and it is usually due to imperfect mixing with Buffer HL or CL, insufficient time to lyse completely, or poor disruption of sample. Check these out in next preparations.

Storage conditions

- Temperature : Room temperature (15~25˚C)
- Humidity : 20~80%

Symbol	Used for	Symbol	Used for
LOT	Batch number		Manufacturer
REF	Catalogue number		Do not re-use
	Consult instructions for use		Date of manufacture
	Caution		Expiry date
	Temperature limit		

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GENEALL BIOTECHNOLOGY CO., LTD.

2024.06

Ver 1.0

GeneAll®

Store at room temperature (15~25°C)
Expiration date : 18 months after manufacture

For research use only

Exgene™ cfDNA SV mini

Cat. No. 129-101
Size: 100 preps

Kit contents

Components	Quantity	Storage
Proteinase K †	48 mg	Room temperature (15~25°C)
PK-Storage buffer †	4 ml	
Buffer HL	40 ml	
Buffer CL	40 ml	
Buffer HW1 (concentrate) *	36 ml	
Buffer HW2 (concentrate) *	20 ml	
Nuclease-free water	20 ml	
Column Type Q w/cap (mini)	100	
2 ml collection tube	100	

Product specifications

Specification	Exgene™ cfDNA SV mini
Type	Spin
Maximum amount of starting samples	1 ml
Maximum loading volume	700 µl
Minimum elution volume	30 µl

† Before using for the first time, Add PK-Storage buffer as indicated on the bottle to one tube of lyophilized Proteinase K, and gently invert to dissolve. Store Proteinase K solution at 4°C. For longer storage, we suggest storing it at -20°C.
** Before using for the first time, add absolute ethanol (ACS grade or better) into Buffer HW1 and HW2 as indicated on the bottle.*

Quality control

All components in the Exgene™ cfDNA SV mini are manufactured and maintained in a state of strict cleanliness.
Rigorous quality control is performed consistently across batches, and only the kits meeting the required standards authorized for delivery.

Storage conditions

All components of Exgene™ cfDNA SV mini should be stored at room temperature (15~25°C) and protected from direct sunlight exposure. During shipment or storage under cool ambient conditions, a precipitate may formed in Buffer HL. In such a case, incubate bottle at 56°C prior to use to dissolve precipitates. Using precipitated buffers will lead to poor DNA recovery.
Exgene™ cfDNA SV mini is guaranteed until the expiration date printed on the product box.

Safety information

The buffers included in Exgene™ cfDNA SV mini contain irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken during handling. Always wear gloves and eye protector, and follow standard safety precautions. In case of contact, wash immediately with plenty of water and seek medical advice.
Buffer HL and HW1 contain chaotropes. It can form highly reactive compounds when combined with bleach. DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Product disclaimer

Exgene™ cfDNA SV mini is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

Preventing contamination

Always wear disposable gloves while handling reagents and samples. The use of sterile tip, tube and other instrument is recommended throughout the procedure.

Intended use

The Exgene™ cfDNA SV mini is designed to extract circulating cell free DNA from plasma, serum, and urine.

Product description

The Exgene™ cfDNA SV mini is designed for the extraction of circulating cell free DNA from plasma, serum, and urine. Utilizing advanced silica-binding technology, this kit efficiently extracts DNA for a wide range of applications.

The process begins by lysing samples in an optimized buffer containing detergent and a lytic enzyme. Under ideal binding conditions, DNA in the lysate binds to a silica membrane, while impurities pass through into a collection tube.

The membranes are then washed with a series of alcohol-containing buffers to remove any traces of proteins, cellular debris, and salts. Finally, the purified DNA is eluted into a clean microcentrifuge tube with deionized water.

This purified DNA can be directly used for various downstream applications such as PCR, qPCR, microarrays and NGS.

Brief protocol

1. Transfer the sample to a 1.5 ml microcentrifuge tube
2. Add 20 µl of Proteinase K solution and 300 µl of Buffer HL
3. Add 300 µl of Buffer CL
4. Incubate at 56°C for 30 min
5. Add 300 µl of chilled absolute ethanol
6. Transfer the mixture into the mini column and centrifuge at 13,000 rpm for 2 min
7. Repeat steps 6
8. Add 600 µl of Buffer HW1 into the mini column and centrifuge at 13,000 rpm for 2 min
9. Add 700 µl of Buffer HW2 into the mini column and centrifuge at 13,000 rpm for 2 min
10. Perform an additional centrifuge for 3 min at full speed
11. Add 30-50 µl of Nuclease-free water into the mini column and incubate for 2 min at room temperature
12. Centrifuge at full speed for 2 min

Protocol

1. **Transfer 300 µl of sample to a 1.5 ml microcentrifuge tube (not provided).**
2. **Add 20 µl of Proteinase K solution (20 mg/ml, provided) and 300 µl of Buffer HL to the sample. Vortex vigorously to mix thoroughly for 30 sec and briefly spin down.**
3. **Add 300 µl of Buffer CL to the sample. Vortex vigorously to mix thoroughly for 30 sec and briefly spin down.**
4. **Incubate at 56°C for 30 min and spin down briefly to remove any drops from inside of the lid.**
5. **Add 300 µl of chilled absolute ethanol (not provided) to the sample, pulse vortex to mix the sample thoroughly for 30 sec, and spin down briefly to remove any drops from inside of the lid.**
6. **Carefully transfer 650 µl of the mixture to the Column Type Q w/cap (mini), centrifuge at 13,000 rpm for 2 min at room temperature, discard the pass-through, and then reinsert the mini column back into the collection tube.**
7. **Repeat the step 6 using remained mixture.**
8. **Add 600 µl of Buffer HW1 to the column. Centrifuge at 13,000 rpm for 2 min at room temperature. Discard the pass-through, and then reinsert the mini column back into the collection tube.**
9. **Add 700 µl of Buffer HW2 to the column. Centrifuge at 13,000 rpm for 2 min at room temperature. Discard the pass-through and then insert the mini column into the new collection tube (provided).**
10. **Centrifuge at full speed for 3 min at room temperature to remove residual wash buffer. Place the mini column into a fresh 1.5 ml microcentrifuge tube (not provided).**
11. **Add 30-50 µl of Nuclease-free water to the center of the membrane into the mini column. Incubate at room temperature for 2 min.**
12. **Centrifuge at full speed for 2 min at room temperature.**

Expected DNA yield

	Amount of starting sample	Yield
Plasma	300 µl	~150 ng
Serum	300 µl	~100 ng
Urine	300 µl	~100 ng

* Note that yields of genomic DNA will vary depending on the sample source and the donor health.