Troubleshooting Guide

Problem	Possible Causes	Suggestions
Low or no recovery column clogging	Incorrect sample storage	Sample should be stored at 4°C or -20°C.
	Too much starting material	Too much starting material lead to inefficient homogenization, followed by poor DNA yields. Reduce the amount of starting material down to 200 mg per prep.
	Insufficient homogenization	Check the step 2 of protocol. It is highly recommended to homogenize the sample thoroughly. Insufficient homogenization may cause low yield.
	Incomplete lysis	Check the step 5 of protocol. Incomplete lysis process leads to low recovery yield. Be sure to mix the pellet in correct volume of Buffer FL by pipetting.
Column clogging	Incomplete homogenization	Be sure to mix the pellet in correct volume of Buffer FL by pipetting. And centrifuge again until the lysate has passed through the membrane.
	Too much starting sample	Too much starting sample can lead to column clogging. Reduce the amount of starting material down to 200 mg per prep.
Low efficiency of DNA amplification	Excess amount of template DNA	An excess amount of template DNA will inhibit a PCR reaction. The template DNA is needed to dilute.
Eluate does not preform well in the downstream application	Residual ethanol remains in eluate	To remove any residual ethanol included in Buffer NW from the mini column membrane, centrifuge again for complete removal of ethanol.

Symbol	Used for	Symbol	Used for
LOT	Batch number		Manufacturer
REF	Catalogue number		Do not reuse
	Consult Instructions For Use		Date of Manufacture
	Caution		Expiry date
	Temperature limitation		

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GENEALL BIOTECHNOLOGY CO., LTD

2022.03
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Ver 1.0

GeneAll®

Store at room temperature
Expiration date : 16 months after manufacture

Exgene™ Stool-Bead DNA

DNA Purification Kit

Description

Exgene™ Stool-Bead DNA kit provides a convenient method for the isolation of total DNA from fresh and frozen stool samples. The optimized buffer system and Glass Bead allow purification of high quality nucleic acids suitable for various applications. Through the procedure, the contained impurities in the samples can easily be removed so that high quality DNA can be purified from host and microbial cells.

From 100 up to 200 mg of stool samples can be processed in 25 minutes. The purification starts with homogenization using Glass Bead. Homogenized supernatant is mixed with lysis buffer. After the lysis, the lysate is mixed with DNA binding buffer and only DNA is then bound on the silica membrane. Following washing step, the bound DNA is eluted by elution buffer.

Purified DNA can be directly applicable in conventional PCR, Real-time PCR, RAPD analysis, AFLP, restriction analysis, molecular diagnosis, and any other downstream applications.

Kit Contents

Components	Quantity 115-151	Storage
Buffer FL	20 ml	Room temperature (15~25˚C)
Buffer PB	15 ml	
Buffer KW30	40 ml	
Buffer NW * †	12 ml	
Buffer EB	15 ml	
2 ml Glass Bead tube	50 ea	
1.5 ml microcentrifuge tube	50 ea	
Column Type G with collection tube (mini)	50 ea	
2 ml collection tube	50 ea	
Proteinase K (24 mg) **	1 ea	
Proteinase K Storage Buffer	1.5 ml	
Manual	1 ea	

** Before using for the first time, add absolute ethanol (ACS grade or better) into Buffer NW as indicated on the bottle.*
† Contains sodium azide as a preservative.
*** After reconstitute Proteinase K with Proteinase K Storage Buffer, Proteinase K mixture should be stored at -20˚C.*

Product Specifications

Exgene™ Stool-Bead DNA Kit	
Type	Spin
Maximum amount of starting sample	200 mg/prep
Preparation time	≥25 min
Maximum loading volume of mini column	700 µl
Elution volume	30~200 µl
Maximum binding capacity	100 µg

Materials Not Provided

Reagent : absolute ethanol (ACS grade or better), 1X PBS
Disposable material : pipette tips, disposable gloves
Equipment : microcentrifuge, vortex mixer, water bath or heating block, suitable protector (e.g., lab coat, goggles, etc.)

Safety Information

The buffers included in the Exgene™ Stool-Bead DNA Kit contain irritants which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken when handling such materials. Always wear gloves and eye protection, and follow standard safety precautions. Buffer FL and Buffer PB contain chaotropic agents, which can form highly reactive compounds when combined with bleach.
DO NOT add bleach or acidic solutions directly to the sample preparation waste.

Quality Control

All components in Exgene™ Stool-Bead DNA Kit are manufactured in strictly clean conditions, and its degree of cleanness is monitored periodically. Quality control is carried out thoroughly from lot to lot, and only the qualified kits are approved to be delivered.

Before Use

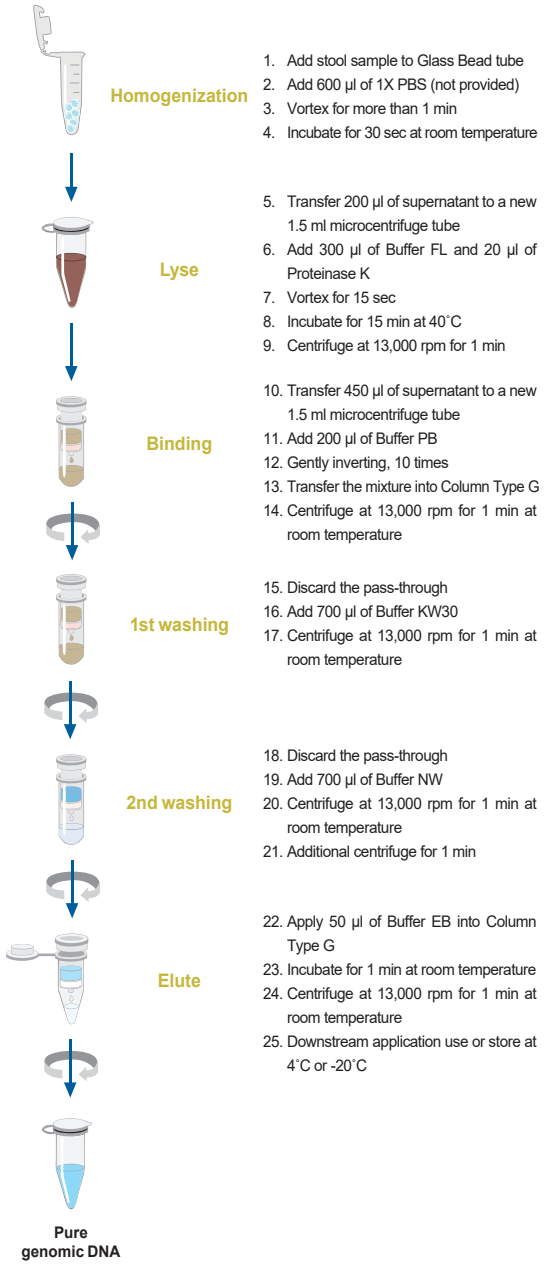
Buffer NW

Buffer NW is supplied as concentrate. Before using for the first time, be sure to add 48 ml of absolute ethanol ethanol (not provided) to obtain a working solution.

Proteinase K

Proteinase K is supplied lyophilized form. The lyophilized Proteinase K can be stored at room temperature (15~25˚C) until the expiration date without affecting performance.
The lyophilized Proteinase K can only be dissolved in PK Storage Buffer. Before using for the first time, be sure to add 1.2 ml of PK Storage Buffer (provided) to obtain a working solution. Reconstituted Proteinase K solution should be immediately stored at -20˚C. The Proteinase K solution is stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date.

Brief Protocol



1. Add stool sample to Glass Bead tube
2. Add 600 µl of 1X PBS (not provided)
3. Vortex for more than 1 min
4. Incubate for 30 sec at room temperature
5. Transfer 200 µl of supernatant to a new 1.5 ml microcentrifuge tube
6. Add 300 µl of Buffer FL and 20 µl of Proteinase K
7. Vortex for 15 sec
8. Incubate for 15 min at 40˚C
9. Centrifuge at 13,000 rpm for 1 min
10. Transfer 450 µl of supernatant to a new 1.5 ml microcentrifuge tube
11. Add 200 µl of Buffer PB
12. Gently inverting, 10 times
13. Transfer the mixture into Column Type G
14. Centrifuge at 13,000 rpm for 1 min at room temperature
15. Discard the pass-through
16. Add 700 µl of Buffer KW30
17. Centrifuge at 13,000 rpm for 1 min at room temperature
18. Discard the pass-through
19. Add 700 µl of Buffer NW
20. Centrifuge at 13,000 rpm for 1 min at room temperature
21. Additional centrifuge for 1 min
22. Apply 50 µl of Buffer EB into Column Type G
23. Incubate for 1 min at room temperature
24. Centrifuge at 13,000 rpm for 1 min at room temperature
25. Downstream application use or store at 4˚C or -20˚C

Protocol

1. **Add 100~200 mg of stool sample to a 2 ml Glass Bead tube (provided).**
2. **Add 0.6~1 ml of 1X PBS (not provided) to the tube and vortex for 1 min or until the stool sample is thoroughly homogenized.**
   It is highly recommended to homogenize the sample thoroughly. Insufficient homogenization may cause low yield.
3. **Incubate for 30 sec at room temperature.**
4. **Transfer 200 µl of the supernatant to the new 1.5 ml microcentrifuge tube (not provided).**
5. **Add 300 µl of Buffer FL and 20 µl of Proteinase K solution to the tube. Vortex vigorously to mix thoroughly.**
6. **Incubate the lysate for 15 min at 40˚C.**
   Vortex the mixture every 3 min while incubating.
7. **Centrifuge at 13,000 rpm at room temperature for 1 min.**
8. **Transfer 450 µl of the supernatant to the new 1.5 ml microcentrifuge tube.**
9. **Add 200 µl of Buffer PB and gently mixing (DO NOT VORTEX).**
10. **Transfer the mixture to the Column Type G (green).**
11. **Centrifuge at 13,000 rpm for 1 min at room temperature. Discard the pass-through and replace the collection tube with new one (provided).**
12. **Add 700 µl of Buffer KW30 to the mini column.**
13. **Centrifuge at 13,000 rpm for 1 min at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**
14. **Add 700 µl of Buffer NW to the mini column.**
15. **Centrifuge at 13,000 rpm for 1 min at room temperature. Discard the pass-through and transfer the mini column back into the collection tube.**
16. **Centrifuge at 13,000 rpm for 1 min at room temperature to remove residual wash buffer. Place the mini column into a new 1.5 ml microcentrifuge tube.**
    Residual ethanol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of Buffer NW.
17. **Add 50~200 µl of Buffer EB to the center of the membrane in the mini column.**
18. **Incubate for 1 min at room temperature.**
19. **Centrifuge at 13,000 rpm for 1 min at room temperature.**

Technical Data

1. Genomic DNA purification

Sample	Yield (ng/µl)	$A_{260/280}$	$A_{260/230}$
Human	**111.3**	1.85	2.09
Bovine	**186.0**	1.85	2.01
Swine	**107.3**	1.85	2.05
Canine	**178.6**	1.85	2.16

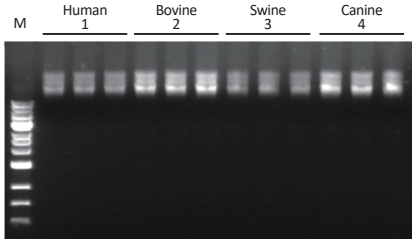


Fig. 1. Result of DNA purification
After eluting genomic DNA with 50 µl Buffer EB, each of 100 ng of DNA were used in DNA electrophoresis.
Lane M : 1 kb DNA Marker
lane 1 : Human stool
lane 2 : Bovine stool
lane 3 : Swine stool
lane 4 : Canine stool

2. Pathogen DNA Real-time PCR

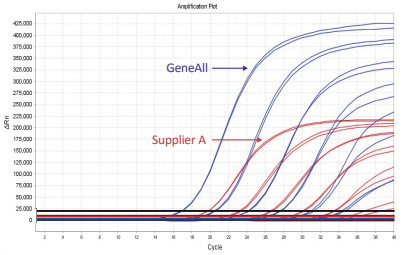


Fig. 2. Result of Salmonella DNA prep & Real-time PCR
Salmonella was amplified with the purified DNA from clinical stool sample as template (5 µl) using salmonella primer & probe.

3. Swine GAPDH Real-time PCR

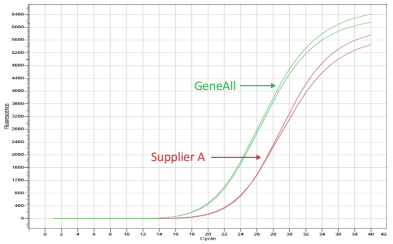


Fig. 3. Swine GAPDH Real-time PCR
The GAPDH was amplified with the purified DNA from Swine stool sample as template (5 µl) using Swine GAPDH primer & probe.