

STOOL DNA ISOLATION MINI KIT

DE-023 & DE-024

The Fisher Molecular Biology Stool DNA Isolation Kit is designed for isolation of high quality total DNA from 50~100 mg of fresh or frozen stool sample. The inhibitors of the downstream application such as polysaccharides, humic acid, phenolic compounds will be removed by utilizing the DNA binding column and the buffer system in this kit. During entire procedure is not required the phenol-chloroform, extractions and can be finished within 60 minutes. The purified DNA is ready for PCR and other downstream applications.

Specifications:

Principle: Spin Column (silica membrane)

Sample: from 50 up to 200 mg

Operation time: < 60 min

Elution volume: 50~200 µl

Kit Components	DE-023s (4 preps_sample)	DE-023 (50 preps)	DE-024 (100 preps)
SDE1 Buffer	1.8 ml	20 ml	40 ml
SDE2 Buffer	1.2 ml	7 ml	14 ml
SDE3 Buffer	1.2 ml	15 ml	30 ml
SDE4 Buffer	3 ml	20 ml	40 ml
Wash Buffer (concentrate) *	1.5 ml	20 ml	35 ml
Elution Buffer	1.5 ml	15 ml	30 ml
Proteinase K (lyophilized) *	1.1 mg	11 mg	11 mg x 2
SDE Mini Column	4 pcs	50 pcs	100 pcs
Collection Tube	8 pcs	100 pcs	200 pcs
Elution Tube	4 pcs	50 pcs	100 pcs
Bead tube	4 pcs	50 pcs	100 pcs
*PREPARATION OF PROTEINASE K Solution and Wash Buffer			
Cat. N°:	DE-023s	DE-023	DE-024
ddH ₂ O volume for Proteinase K Solution	0.11 ml	1.1 ml	1.1 ml
ethanol volume for Wash Buffer	6 ml	80 ml	140 ml

1. Buffers provided in this system contain irritants. Wear gloves and lab coat when handling these buffers.
2. Check SDE1 Buffer before use, Warm SDE1 Buffer at 60 °C for 10 minutes if any precipitate formed.
3. Add required sterile ddH₂O to Proteinase K tube to make a 10 mg/ml stock solution. Vortex and make sure that Proteinase K has been completely dissolved. **Store the stock solution at 4 °C.**
4. Add indicated volume of ethanol (96-100%) to Wash Buffer before use.
5. Prepare a heating block or a water bath to 60 °C. If DNA is isolated from gram positive bacteria, prepare a heating block or a water bath to 95 °C for another incubation.
6. All centrifuge steps are done at full speed (~18,000 x g) in a microcentrifuge.
7. Preheat Elution Buffer or ddH₂O to 60 °C for elution step.

General Protocol:

Please Read Important Notes Before Starting Following Steps.

1. Add up to 200 mg of Stool Sample to a Bead Tube (provided), then place on ice. --If the sample is liquid, transfer 200 μ l of stool sample into a Bead Tube.
2. Add 300 μ l of SDE1 Buffer and 20 μ l of proteinase K (10 mg/ml) to the sample. Vortex at maximum speed for 5 minutes. Incubate the sample mixture at 60 °C for 20 minutes and vortex the sample for every 5 minutes during the incubation.

-- Make sure stool sample is homogenized completely.

-- For isolation of DNA from gram positive bacteria, do a further incubation at 95 °C for 5 minutes after proteinase K lysis.
3. Briefly spin the tube to remove drops from the inside of the lid.
4. Cool down the sample mixture and add 100 μ l of SDE2 Buffer. Mix well by vortexing and incubate the sample mixture on ice for 5 minutes.
5. Centrifuge at full speed (~18,000 x g) for 5 minutes.
6. Carefully transfer the supernatant to a 1.5 ml microcentrifuge tube (not provided) and discard the stool pellet.
--Avoid pipetting any debris and pellet.
7. Add 200 μ l of SDE3 Buffer. Mix well by vortexing and incubate the sample mixture at room temperature for 2 minutes. --Note: SDE3 Buffer must be suspended completely by vigorously vortexing before every using. -- Cut off the end of 1 ml tip to make it easier for pipetting the SDE3 Buffer.
8. Centrifuge at full speed for 2 minutes.
9. Carefully transfer 250 μ l of supernatant to a 1.5 ml microcentrifuge tube (not provided). --Avoid pipetting any debris and pellet.
10. (Optional) If RNA-free DNA is required, add 1 μ l of 100 mg/ml RNase A (not provided). Mix well and incubate the sample mixture at room temperature for 2 min.
11. Briefly spin the tube to remove drops from the inside of the lid.
12. Add 250 μ l of SDE4 Buffer and 250 μ l of ethanol (96~100%). Mix thoroughly by pulse-vortexing.
13. Place a SED Column into a Collection. Transfer all of the sample mixture to the SDE Column. Centrifuge at full speed for 1 min and discard the flow-through then place the SDE Column into a new Collection Tube.
14. Add 750 μ l of Wash Buffer (ethanol added) to the SDE Column. Centrifuge at full speed for 1 min then discard the flow-through. Return the SDE Column back to the Collection Tube. --Make sure that ethanol (96~100%) has been added into Wash Buffer when first use.
15. Repeat step 14.
16. Centrifuge at full speed for an additional 3 min to dry the SDE column.
--Important step! This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.
17. Place the SDE Column into a 1.5 ml microcentrifuge tube (not provided). Add 50 ~ 200 μ l of preheated Elution Buffer or ddH₂O to the membrane center of the SDE Column. Stand the SDE Column for 2 min at room temperature.
--Important step! For effective elution, make sure that the Elution Buffer or ddH₂O is dispensed onto the membrane center and is absorbed completely.
18. Centrifuge at full speed for 1 min to elute DNA.

Troubleshooting

Problem	Possible reasons	Solutions
Low or no yield of genomic DNA		
	Sample stored incorrectly	Store the stool sample at -20 °C.
	Low amount of cells in the sample	Increase the sample size
Poor cell lysis		
	Poor cell lysis because of insufficient beads beating time	Extend the beads beating time.
	Poor cell lysis because of insufficient mixing with SD1, SD2 Buffer and Proteinase K	Mix the sample mixture immediately thoroughly by pulse - vortexing after adding SD1, SD2 Buffer and proteinase K solution.
	Poor cell lysis because of insufficient Proteinase K activity	Use a fresh or well-stored Proteinase K stock solution.
Insufficient binding of DNA to column's membrane		
	Ethanol is not added into sample lysate before DNA binding	Make sure that the correct volumes of ethanol (96- 100 %) is added into the sample lysate before DNA binding.
	Ethanol and sample lysate did not mix well before DNA binding	Make sure that Ethanol and sample lysate have been mixed completely before DNA binding
Incorrect preparation of Wash Buffer W1/W2		
	Ethanol is not added into Wash Buffer when first use	Make sure that the correct volumes of ethanol (96- 100 %) is added into Wash Buffer when first use.
	The volume or the percentage of ethanol is not correct for adding into Wash Buffer	Make sure that the correct volumes of ethanol (96- 100 %) is added into Wash Buffer when first use.
Elution of DNA is not efficient		
	pH of water (ddH ₂ O) for elution is acidic	Make sure the pH of ddH ₂ O is between 7.0-8.5. Use Elution Buffer (provided) for elution .
	Elution Buffer or ddH ₂ O is not completely absorbed by column membrane	After Elution Buffer or ddH ₂ O is added, stand the SD Column for 5 min before centrifugation.
Poor quality of genomic DNA		
A260/A280 ratio of eluted DNA is low	Poor cell lysis	
	Poor cell lysis because of insufficient beads beating time	Extend the beads beating time.
	Poor cell lysis because of insufficient mixing with SD1 buffer, SD2 Buffer and Proteinase K	Mix the sample mixture immediately thoroughly by pulse - vortexing after adding SD1, SD2 Buffer and proteinase K solution.
	Poor cell lysis because of insufficient Proteinase K activity	Use a fresh or well-stored Proteinase K stock solution.
A260/A280 ratio of eluted DNA is high	A lot of residual RNA in eluted DNA	Add 8 µl of RNase A (50 mg/ml) to the eluate and incubate at 37 °C for 10 minutes. After incubation, add 200 µl of SD2 Buffer and 200 µl of ethanol (96~100%), mix well by plus -vortexing. Then follow the general Protocol starting from step 7.