

ODP308

Blood DNA Kit

For isolation of genomic DNA from mammals, fish, and avian whole blood.



ISO 13485:2016 ISO 9001:2015

Blood DNA Kit

Spin Column
(ODP308)

Kit contents

Components	50 Preps
Buffer GA	10mL
Buffer GB	10mL
Buffer GD	13mL
Buffer PW	15mL
Buffer TE	10mL
Proteinase K (20mg/mL)	1mL
Spin column CB3 (2mL) Collections Tubes	50

Storage

Blood DNA Kit could be stored dry at room temperature (15 -25°C) for up to 12 months without any diminution in performance and quality. For long-term storage, the kit could be stored at 2-8°C.

Proteinase K should be stored at -20°C upon receipt of the kit.

Introduction

Blood DNA Kit is based on silica membrane technology and special buffer system for genomic DNA extraction from various kinds of blood samples. The spin column CB3, made of new type silica membrane can bind DNA optimally on given salt and pH conditions. Simple centrifugation completely removes contaminants and enzyme inhibitors such as proteins and divalent cations. Extracted DNA is eluted in low-salt buffer or water, ready to use in down-stream applications like restriction analysis, PCR analysis, Southern blotting and cDNA library.

Precautions

1. Repeated freezing and thawing of stored samples lead to lower DNA yield.
2. If precipitate is observed in Buffer GB and Buffer GD, warm up the buffer to 56°C until the precipitate dissolves completely.
3. All centrifugation steps should be carried out in a conventional table-top microcentrifuge at room temperature (15–25°C).
4. Ensure thorough mixing of blood by inverting the tube before the assay.

Materials required but not provided

1. 1.5mL microcentrifuge tubes
2. Ethanol (96-100%)

Protocol

Ensure that Buffer GD and Buffer PW have been added with appropriate volume of ethanol (96-100%) as indicated on the bottle and mix thoroughly.

Note: It is advised to reconstitute ONLY required volume of Buffer GD with ethanol (96-100%) as reconstituted Buffer GD will precipitate on long term storage.

1. Pipette 20 μ L Proteinase K to a 1.5mL microcentrifuge tube.
 2. Preparation of samples,
 - a) Non-nucleated: Pipette 200 μ L sample to the microcentrifuge tube, and mix thoroughly by pulse-vortexing. Then add 180 μ L of Buffer GA and mix thoroughly by vortexing. If the volume is less than 200 μ L, adjust volume to 200 μ L with Buffer GA. If the sample volume is more than 200 μ L, e.g., 300 μ L - 1mL, add 3 times volume Red Blood Cell Lysis Buffer (Cat# ORT122, not provided in the kit) to the sample and mix by inversion. Keep the tube at room temperature (15–25°C) for 5 minutes, and centrifuge at 12,000 rpm (~13,400 \times g) for 1 minute, discard the supernatant, pipette 180 μ L Buffer GA and mix by vortexing for 30 seconds.
 - b) Nucleated: Add 5-20 μ L anticoagulated blood; adjust volume to 100 μ L with Buffer GA and mix by vortexing for 30 seconds.
- Note: If RNase-free genomic DNA is required, add 4 μ L RNase A (10mg/mL; Cat# ORT405, not provided). Mix thoroughly by vortexing for 15 seconds and incubate for 5 minutes at room temperature (15–25°C).**
3. Incubate at 56°C for 10 minutes to yield a homogenous solution after adding 180 μ L Buffer GA to the samples.
 4. Add 200 μ L Buffer GB to the sample, mix thoroughly by vortexing for 30 seconds.
- Note: Precipitates are expected, but it will not interfere with the extraction.**
5. Add 200 μ L ethanol to the sample, mix thoroughly by vortexing for 60 seconds.
- Note: Precipitate formed in the earlier step will be dissolved.**
6. Pipette the mixture from step 5 into the spin column CB3 (in a 2mL collection tube) and incubate at room temperature for 5-10 minutes. Centrifuge at 12,000 rpm (~13,400 \times g) for 45 seconds and discard flow-through. Place the spin column CB3 into the collection tube.
 7. Add 500 μ L Buffer GD to spin column CB3, and centrifuge at 12,000 rpm (~13,400 \times g) for 30 seconds, then discard the flow-through and place the spin column into the collection tube.
- Note: It is advised to reconstitute ONLY required volume of Buffer GD with ethanol (96-100%) as reconstituted Buffer GD will precipitate on long term storage.**
8. Add 700 μ L Buffer PW to spin column CB3, and centrifuge at 12,000 rpm (~13,400 \times g) for 30 seconds. Discard the flow-through and place the spin column into the collection tube.
 9. Repeat step 8.

10. Centrifuge at 12,000 rpm (~13,400 ×g) for 2 minutes to dry the membrane completely and air-dry the column at room temperature for 2 minutes.

Note: Residual ethanol of Buffer PW may have some effect in downstream applications.

11. Place the spin column CB3 in a new clean 1.5mL microcentrifuge tube, and pipette 50-200µL Buffer TE directly to the center of the membrane. Incubate at room temperature (15–25°C) for 2-5 minutes, and then centrifuge for 2 minutes at 12,000 rpm (~13,400 ×g).

Note: If the volume of eluted buffer is less than 50µL, it may affect recovery efficiency. For long-term storage of DNA, eluting in Buffer TE and storing at –20°C is recommended, since DNA stored in water may be subjected to acid hydrolysis. To increase the DNA yield, introduce the eluted Buffer TE to the spin column CB3 and centrifuge for 2 minutes at 12,000 rpm (~13,400 ×g).

Performance

Sample	Yield (µg from 10µL whole blood)			
	Elution 1	Elution 2	Elution 3	Total
Fish	1.83	0.40	0.06	2.29
Chicken	0.90	0.89	0.43	2.22
Mammals	0.62	0.185	0.01	0.815

Total DNA yield: Genomic DNA isolated from whole blood samples using Blood DNA Kit. Each elution was done with 200µL Buffer TE.

Note: Total DNA yield from mammalian whole blood is lower since they have a nucleated RBCs.