For research purpose only. Not for use in diagnostic procedures for clinical purposes. For IN VITRO USE ONLY.

ISO 9001/14001 Certified Company

G-DEX™IIb Genomic DNA Extraction Kit (for Blood)

Cat. No. 17241 200 Tests

DESCRIPTION

The G-DEX™IIb Genomic DNA Extraction Kit (for Blood) produces pure and high yield of superior-quality DNA from blood and buffy coat samples, collected in all common anti-coagulants (EDTA, citrate, heparin). Samples from 0.1 to 20 ml can be obtained 4.6 μg ~ 813 μg DNA in about an hour or less.
The easy-to-use procedure can overcome the time-consuming, utilization of toxic organic solvent low solubility and low yield.

CHARACTERISTICS

- Variety : Extract from a wide range of biological samples
- Rapid isolation of Genomic DNA : 20 ~ 60 min
- Easy-to-use : Lysis → protein removal → DNA precipitation → DNA hydration
- High yield : Recovers up to 6.5 μg (300 μl blood)

APPLICATION

- Genotyping (SNP)
- Pharmacogenomic research
- Oncology research

KIT CONTENTS AND STORAGE

Label	Description	Contents
RBC Lysis Solution	RBC Lysis	180 ml
Lysis Buffer	Cell lysis buffer	60 ml
Protein PPT Buffer	Protein precipitation buffer	25 ml
RNase A(lyophilized powder)	Dissolve with 0.75 ml of pure DW	3 mg
DNA Rehydration Solution	DNA Rehydration buffer	25 ml

1) All components are stored at room temperature. The term of validity is marked on the box
2) **Lyophilized RNase A** : Dissolve the RNase A in 0.75 ml of pure D.W. to each vial. The lyophilized RNase A can be stored at room temperature (15–25°C) until the expiration date without affecting performance. The lyophilized RNase A can only be dissolved in D.W.; dissolved RNase A should be immediately stored at -20℃. The RNase A solution is stable at -20°C for up to 24 months and 20 times frozen-thawing until the kit expiration date.

ADDITIONAL REQUIRED EQUIPMENT

- Agarose(iNtRON, 32034); scalpel
- Gel running buffer: TAE buffer or TBE buffer Electrophoresis Sterile
- Absolute ethanol
- Isopropanol (2-propanol)
- Standard tabletop microcentrifuge
- Microcentrifuge tubes, sterile (1.5 ml)

NOTICE BEFORE USE

▪ WBC NUMBER BY BLOODS

According to the volume of whole blood, it shows about the present WBC number.

Blood	WBC (cells/ml)	Blood	WBC (cells/ml)
100 μl	7×10^5 ~ 8×10^5	5ml	3.5×10^7 ~ 4.0×10^7
300 μl	2.0×10^6 ~ 2.5×10^5	10ml	7×10^7 ~ 8×10^7
1ml	7×10^6 ~ 8×10^6	20ml	1.4×10^8 ~ 1.6×10^8

▪ TUBE SELECTION

Bloods	Tube
100 μl ~ 500 μl	1.5 ml tube
600 μl ~ 4 ml	15 ml tube
5 ml ~ 20 ml	50 ml tube

According to the blood volume, it is easy to select the proper sample tubes.

REPRESENTATIVE PROTOCOL

This method given in the following protocol is suitable for isolation of DNA from human whole blood. The protocol can be adapted for DNA isolation from blood samples by scaling reagent volume of blood used (see table 1).

PROTOCOL

1. Add 300 μl whole blood (or bone marrow) to a 1.5 ml tube containing 900 μl RBC Lysis Solution. Mix thoughly by voltexing and incubate for 5 minutes at room temperature. Invert again at least once during the incubation.
2. Centrifuge at 10,000 x g for 1 minutes. Remove supernatant except the white cell pellet and remain about 50 ~ 100 μl of the remnant.
   Note : Remain the little volume of solution. It helps cell pellet resuspending. The solution volume in the residual liquid is not important.
   Note : When you use table top centrifuge e, use the maximum rpm.
3. Vortex the tube vigorously to resuspend the cells.
   Note : In order to resuspend the pellet completely, vortex carefully.
4. Add 300 μl Cell Lysis Solution to the resuspended cells and do pipetting up and down to lyse the cells.
   Note : If cell clumps are visible after mixing, incubate at 37℃ or room temperature until the solution is homogeneous.
5. (Optional) Add 1.5 μl RNase A Solution to the cell for lysate and incubate at 37℃ for 15~30 minutes.
   Note : After adding the RNase A at 37℃,it is very efficient by inverting the tube sometimes.
   Note : After the DNA is dissolved in DNA Hydration buffer, add the RNase A. In general, it is spontaneously removed when the genomic DNA is extracted from blood.
6. Chill sample to room temperature. Add 100 μl PPT buffer to the cell lysate. And vortex vigorously at high speed for 20 seconds.
   Note : In order to remove the protein contamination , in some case, put the sample to the ice during 5 min.
7. Centrifuge at 13,000 ~ 16,000 x g for 3 ~ 5 minutes. The precipitated proteins will form a tight white pellet.
   Note : If the protein pellet is not tight, repeat Step 6 followed by incubation on ice for 5 minutes and then repeat Step 7.
8. Transfer to the 300 μl of supernatant containing the DNA (leaving behind the precipitated protein pellet) into a 1.5 ml tube. Add 300 μl 100% Isopropanol (2-propanol) and mix the sample by inverting gently several times.
   Note : When the low DNA yield, add the glycogen(200 mg/ml) which is 1/100 volume of Cell Lysis Buffer before adding the isopropanol . Representative Isopropanol (2-propanol) volume is 90 ~ 95% of supernatant. which transferred lysate volume. It is good to add Isopropanol (2-propanol) about the same volume of supernatant.
9. Centrifuge at 13,000 ~ 16,000 x g for 1 minutes; the DNA will be visible as a small white pellet.
10. Pour off supernatant and drain tube briefly on clean absorbent paper. Add 1 ml 70% Ethanol and invert the tube several times to wash the DNA pellet. And Centrifuge at 13,000 ~ 16,000 x g for 1 minute. Carefully pour off the ethanol. Otherwise, Pellet may be loose. So, pour slowly and watch pellet.
11. Invert and drain the tube on the clean absorbent paper and allow to air dry for 10 ~ 15 minutes.
    Note : When discard the supernatant, be careful not to discard the DNA pellet. Avoid the over dry because too much dried DNA is not dissolved very well in DNA Hydration buffer.
12. Add 150 μl DNA Rehydration Buffer.
    Note : When the high DNA yield, it is very difficult to dissolve the DNA . In this case, add the proper volume of the solution.
13. Rehydrate DNA by incubating at 65℃ for 30 min ~ 60 min or at 4℃ for overnight culture. If possible, tap tube periodically to aid in dispersing the DNA.
14. For long-term storage, collected DNA is stored at -20℃ or -80℃.
15. Measure the DNA purity O.D 260:280 ratio.

TECHNICAL INFORMATION

◎ Experimental Guideline and Typical yield

Table 1 shows the solution volume according to the blood volume. For the most suitable efficiency, refer and follow under the table.

Table 1. Solution Amounts According to Blood Amounts

Blood	RBC Lysis	Cell Lysis	RNase A	PPT Buffer	Rehyd. Buffer	Yields (µg)
100 µl	300 µl	60 µl	0.5 µl	20 µl	25 µl	4.6 ± 0.8
300 µl	900 µl	300 µl	1.5 µl	100 µl	50 µl	6.5 ± 3.3
600 µl	1.8 ml	600 µl	1.0 µl	200 µl	75 µl	17.1 ± 13.8
1 ml	3 ml	1 ml	5.0 µl	330 µl	100 µl	27.4 ± 11.8
2 ml	6 ml	2 ml	10 µl	660 µl	200 µl	59.7 ± 35
3 ml	9 ml	3 ml	15 µl	1.0 ml	300 µ	106 ± 58
4 ml	12 ml	4 ml	20 µl	1.3 ml	400 µl	144 ± 61
5 ml	15 ml	5 ml	25 µl	1.6 ml	500 µl	192 ± 89
6 ml	18 ml	6 ml	30 µl	2.0 ml	600 µl	241 ± 124
7 ml	21 ml	7 ml	35 µl	2.3 ml	700 µl	289 ± 185
8 ml	24 ml	8 ml	40 µl	2.6 ml	800 µl	339 ± 198
9 ml	27 ml	9 ml	45 µl	3.0 ml	900 µl	381 ± 215
10 ml	30 ml	10 ml	50 µl	3.3 ml	1 ml	426 ± 223
20 ml	60 ml	20 ml	55 µl	3.6 ml	2 ml	813 ± 355

1) In case of rehydration buffer, add the solution over 1.5 ~ 3 times compared with the standard when the DNA pellet is not dissolved very well.
2) Representative Isopropanol (2-propanol) volume is 90~95% of supernatant which transferred lysate volume. It is good to add Isopropanol (2-propanol) with 1 volume of supernatant.
3) If the DNA yield is low, add the glycogen for increasing the yield of DNA recovery (glycogen is a highly purified polysaccharide that can be used as a carrier for nucleic acid PPT).
4) People blood is used in this DNA yield measurement, it depends on blood conditions, experimental conditions, and user.

◎ DNA Stability For Long Term Preservation

Every extracted DNA pellet were completely dissolved with rehydration buffer then preserved under freezing condition (-22~-18 ℃). After preserving period, the DNAs were analyzed for agarose gel electrophoresis. The figure shows that the DNAs are stable under long term preservation condition.

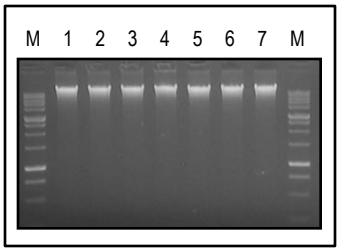

Figure 1. Agarose gel electrophoresis analysis
Lane M , 1kb DNA Ladder; **lane 1**, fresh gDNA; **lane 2**, preserved DNA for 1 week; **lane 3**, preserved DNA for 2 weeks; **lane 4**, preserved DNA for 1 month; **lane 5**, preserved DNA for 3 months; **lane 6**, preserved DNA for 6 months; **lane 7**, preserved DNA for 1 year

The DNAs were diluted serially then applied to PCR analysis. The PCR sensitivity was shown similar entire testing group. Those mean the preserved DNAs are stable for long term preservation without DNA degradation.

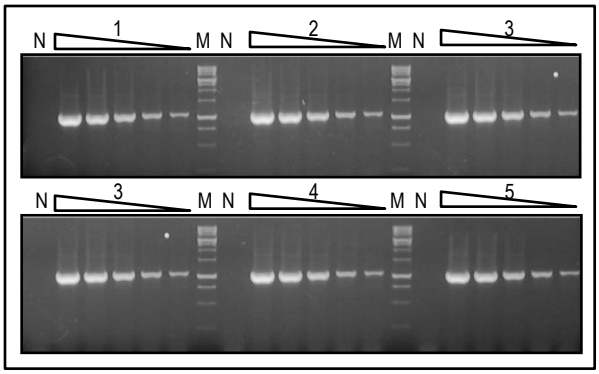

Figure 2. PCR Sensitivity Test For Long Term Preservation
Lane M , 100 bp DNA Ladder; **lane N**, negative control (w/o template); **panel 1**, fresh gDNA samples; **panel 2**, preserved DNA for 1 month; **panel 3**, preserved DNA for 3 months; **panel 4**, preserved DNA for 6 months; **panel 5**, preserved DNA for 1 year

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
Cells are incompletely lysed	Cell clumps were present after adding Cell Lysis Solution	- Cells may clump if cells are not completely resuspended prior to addition of Cell Lysis Solution. To lyse the cells in the clumps, incubate sample at either 37°C with periodic mixing until the solution is homogeneous. Cell clumps may be dispersed more quickly by adding Proteinase K and incubating at 65°C until cells are completely lysed (1 h to overnight).
	Too many cells were used	- Reduce the amount of sample or increase the lysis buffer
Protein pellet soft, loose, or absent	Sample was not cooled sufficiently before adding PPT Buffer	- Revortex the sample for 20 sec to mix the PPT Buffer uniformly with the cell lysate. - Incubate sample on ice for 5–15 min to facilitate formation of a tight pellet. - Centrifuge according to the protocol to pellet the precipitated proteins.
	PPT Buffer was not mixed uniformly with the cell lysate	- Be sure to vortex vigorously for the full 20 sec as specified in the protocol.
Samples are slow to rehydrate	Samples were not mixed during the hydration step The DNA pellet was dried too long prior to adding DNA Rehydration Buffer	- DNA pellets that are too dry will require a longer time to rehydrate completely. To rehydrate, incubate at 65°C for 1 h and at room temperature overnight. DNA in DNA Rehyd. Buffer can be stored at room temperature for up to 1 year. - Using heat or vacuum to dry DNA pellet is not recommended.
A260/A280 too high	RNA contamination	- Increase RNase incubation time in lysate from 15 min to 30–60 min.
Red blood cells in the sample were not completely lysed	Higher than average number of red blood cells in the sample	- Repeat the incubation with RBC Lysis Solution to lyse the remaining RBC. Add 3 volumes RBC Lysis Solution for each volume of sample, incubate for 10 min at room temperature, and then centrifuge according to the original protocol followed.

RELATED PRODUCTS

Product name	Cat. No.
Viral Gene-spin™ Viral DNA/RNA Extraction Kit	17151
SiZer™-1000 plus DNA Marker Solution	24075
G-spin™ Total DNA Extraction Mini Kit	17045/17046