G-DEX™ IIc Genomic DNA Extraction Kit

Representative Protocol For 1~2 × 10^6 Cells

Protocol

Notice Before Use

•See the tables for the best result:
[Table 1] Solution Amounts According to Cell Number
[Table 2] Appropriate Tube Selection Guide
[Table 3] Recommended Cell Number
• Recommended cell number is based on HeLa cell. Please refer [Table 3] and it can be modified for adhesive cells.
•This protocol is for genomic DNA extraction from cultured cell (1~2 x 10^6 cells).

Kit Contents (CAT. NO. 17231)

Lysis Buffer 60ml	PPT Buffer 25ml	RNase A Sol. 300µl	DNA Rehyd. Buffer 25ml

Representative Protocol

This protocol is for extraction of genomic DNA from 1~2 x 10^6 cells and the recommended condition can be depended on the number of cells (under-lined). Please, refer the [Table 1].

1st Cell Lysis Step
1.Prepare 1~2 x 10^6 cells in PBS into 1.5 ㎖ tube.
2,Centrifuge at 13,000~16000 x g for 5 sec (RT), then remove supernatant except 5~10 ㎕.
[Note 1] The remained solution can help to resuspend the pellet easily.
[Note 2] It is recommended to use the tabletop centrifuge at maximum rpm.
3.Resuspend the pellet completely. [Note 3] Leaving no cell clumps.
4.Add 150 ul of Cell Lysis Buffer into the tube and lysis the cells by pipetting 5-20 times.
[Note 4] If there are some clumps, incubate the tube at 37 ℃ until the clumps are dissolved completely.

2nd RNase Treatment Step
1.Add 1 ㎕ of RNase A Solution into the tube, and incubate at 37℃ for 15~30 min.
[Note 5] It is recommended to mix occasionally during the incubation.
[Note 6] RNase A solution can be added after resolving DNA in DNA Hydration Buffer .

3rd Protein Precipitation Step
1.Cool down until room temperature and add 50 ㎕ of PPT Buffer, then vortex for 20 sec.
[Note 7] This step is essential to remove protein. Samples can be incubated on ice if necessary.
2.Centrifuge at 13,000~16,000 x g for 3~5 min,this step for the removing the protein pellet.
[Note 8] If the protein pellet is not visible, incubate the tube on ice for 5 min and centrifuge it again.

4th DNA Precipitation Step
1.Transfer the aqueous phase in to a new tube and add 150 ㎕ of isopropanol (2-propanol, 100%), then invert tubes several times.
[Note 9] If DNA yield poorly, add 1/100 ㎕ of glycogen(20 ㎎/㎖) before adding isopropanol. It is recommended to adjust aqueous phase volume and 2-propanol in the ratio of 1:1.
2.Centrifuge at 13,000~16,000 x g for 1 min to gain DNA pellet.
3.Discard the supernatant and keep the tube on the paper towel to dry the pellet.
Add 150 ㎕ of 70% ethanol and invert several times, then centrifuge at 13,000~16,000 x g for 1 min.
[Note 10] 500 ㎕~ 1 ㎖ of 70% ethanol can be used.
4.Discard aqueous phase carefully and the pellet should be air-dried for 10~15 min.
[Note 11] Overdrying the pellet may make the DNA difficult to dissolve again.

5th DNA Rehydration Step
1.Add 150 ㎕ of DNA Rehydration buffer and dissolve the pellet.
[Note 12] The amount of DNA Rehydration buffer can be adjusted by DNA concentration. Refer the table 1.
2. If the pellet is over-dried, incubate at 65 ℃ for 30~60 min or 4 ℃ for O/N.
3.Extracted DNA concentration and purity (A_{260}/A_{280}) should be determined by UV spectrophotometer.
4.Store at –20 ℃ (short term) or –80 ℃ (long term).

Protocol

Tablet 1 Solution Amounts According to Cell Number (K562)

•We recommend this solution amounts table for the best result.

Cell Number (cells)	Cell Lysis	RNase A	PPT Buffer	Isopropanol (not supplied)	70% EtOH (not supplied)	Rehydration buffer	DNA Yields
100 ~ 10,000	60 µl	0.5 µl	20 µl	60 µl	60 µl	25 µl	43 ± 6 ng
10,000 ~ 100,000	100 µl	0.5 µl	33 µl	100 µl	100 µl	25 µl	45 ~ 910 ng
1.0 ~ 9.0 ×10^5	150 µl	1.0 µl	50 µl	150 µl	150 µl	50 µl	1 ~ 12 µg
1.0 ×10^6	150 µl	1.0 µl	50 µl	150 µl	150 µl	100 µl	13 ± 4 µg
1.0 ~ 2.0 ×10^6	300 µl	1.5 µl	100 µl	300 µl	300 µl	150 µl	9 ~ 17 µg
3.0 ~ 5.0 ×10^6	600 µl	3.0 µl	200 µl	600 µl	600 µl	200 µl	20 ~ 35 µg
5.0 ×10^6	600 µl	3.0 µl	200 µl	600 µl	600 µl	200 µl	35 ± 4 µg
6.0 ~ 9.0 ×10^6	750 µl	4.5 µl	250 µl	750 µl	750 µl	300 µl	33 ~ 44 µg
1.0 ×10^7	1.5 ml	7.5 µl	500 µl	1.5 ml	1.5 ml	500 µl	48 ± 6 µg
1.0 ~ 2.0 ×10^7	3.0 ml	15 µl	1 ml	3.0 ml	3.0 ml	500 µl	46 ~ 134 µg
3.0 ~ 5.0 ×10^7	6.0 ml	30 µl	2 ml	6.0 ml	6.0 ml	600 µl	84 ~ 331 µg
5.0 ×10^7	6.0 ml	30 µl	2 ml	6.0 ml	6.0 ml	600 µl	328 ± 19 µg
6.0 ~ 9.0 ×10^7	10.0 ml	50 µl	3.3 ml	10.0 ml	10.0 ml	800 µl	306 ~ 470 µg
1.0 ~ 2.0 ×10^8	15.0 ml	75 µl	5 ml	15.0 ml	15.0 ml	1 ml	560 ~ 1,700 µg

(1) Isopropanol and 70% ethanol are not supplied.
(2) K562 cell line is used for this table and other cell line may be different slightly.
(3)The amount of isopropanol is based on 90 ~ 95% of aqueous phase. It is recommended to adjust aqueous phase volume and 2-propanol in the ratio of 1:1.
(4)More Rehydration (1.5~3 times) buffer is required to redissolve the pellet. Note that DNA concentration should be considered.
(5)If DNA yield poorly, add 1/100 ㎕ of glycogen(20 ㎎/㎖) before adding isopropanol. Glycogen is a highly purified polysaccharide that can be used as a carrier for nucleic acid PPT.

Table 2 :Appropriate Tube Selection Guide

Cell Number	Tube
100 ~ 10,000	0.6 ml tube
10,000 ~ 5.0 × 10^6	1.5 ml tube
6.0 ~ 9.0 × 10^6	2.0 ml tube
1.0 × 10^7 ~ 5.0 × 10^7	15 ml tube
6.0 × 10^7 ~ 2.0 × 10^8	50 ml tube

There are many kinds of dishes and flasks for the cell culture, so we describe them to the table 3 ,it can be shown the proper seeding density and confluency depending on the cell number. This table is based on the HeLa Cell.

Table 3 Useful Numbers for Cell Culture

	Surface Area (mm^2)	Seeding Density	Cells at Confluency
Dishes			
35 mm	962	0.3 × 10^6	1.2 × 10^6
60 mm	2,827	0.8 × 10^6	3.2 × 10^6
100 mm	7,854	2.2 × 10^6	8.8 × 10^6
150 mm	17,671	5.0 × 10^6	20.0 × 10^6
Plates			
6-well	962	0.3 × 10^6	1.2 × 10^6
12-well	401	0.1 × 10^6	0.4 × 10^6
24-well	200	0.5 × 10^6	0.2 × 10^6
Flasks			
T-25	2,500	0.7 × 10^6	2.8 × 10^6
T-75	7,500	2.1 × 10^6	8.4 × 10^6
T-160	16,000	4.6 × 10^6	18.4 × 10^6

Related Products

ITEM	CAT. NO.	SIZE
G-spin™ Genomic DNA Kit (For Cell/Tissue)	17041	50 col.
G-spin™ Genomic DNA Kit (For Blood)	17111	50 col.
G-spin™ Genomic DNA Kit (For Bacteria)	17121	50 col.
G-spin™ Genomic DNA Kit (For Plant)	17191	50 col.
Viral Gene-spin™ Viral DNA/RNA Extraction Kit	17151	50 col.
RNA-spin™ Total RNA Extraction Kit	17211	50 col.