G-DEX™ IIc Genomic DNA Extraction Kit [for Cell/Tissue]

The Instruction Manual for genomic DNA Extraction from tissues and cells using alcohol precipitation method.

RUO REF 17231 Σ 300 RT

DESCRIPTION

The **G-DEX™IIc Genomic DNA Extraction Kit (for Cell/Tissue)** is a rapid and efficient method for isolation of high molecular weight genomic DNA from gram negative bacteria, animal cells and tissues of all types. For the most samples, it takes just 5 steps and less than 1 hour. All samples can obtain high yields of superior-quality DNA. Also, sample sizes can range from a single cell to 1 gram of tissue. It is suitable for PCR, DNA hybridization, genomic DNA library construction, and other applications.

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 20 μg (1 ~ 2 x 10^6 Cells), 35 μg (~20 mg Tissue)
- **High purity** : DNA ratio ($OD_{260/280}$) = 1.9 ~ 2.1 (Free from contaminants)

KIT CONTENTS

Label	Description	Contain
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 Buffer	DNA Rehydration Buffer	25 ml
Instruction Manual		1 ea

STORAGE AND STABILITY

- All components are stored at room temperature. The term of validity is marked on the box
- **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°C. The RNase A solution is stable at -20 °C for up to 24 months and 20 times frozen-thawing until the kit expiration date.

PRODUCT USE LIMITATIONS

G-DEX™IIc Genomic DNA Extraction Kit (for Cell/Tissue) is developed, designed, and sold for **research purpose only**. They are **not to be used for human or animal diagnostic or therapeutic uses.** Do not use internally or externally in humans or animals. Be careful in the handling of the products.

APPLICATIONS

- Genotyping (SNP)
- Pharmacogenomic research
- Oncology research

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

All products undergo extensive quality control test and are warranted to perform as described when used correctly. Immediately any problems should be reported. Satisfaction guarantee is conditional upon the customer providing full details of the problem to iNtRON within 60 days, and returning the product to iNtRON for examination.

PRECAUTION AND NOTICE BEFORE USE

- The mixing condition is different by adding cell number, (see the table 1). The table 3 shows that the tube is used according to the cell number.
- Also, see the table 2. It shows present cell number according to the flask in adherent cell number.
- If you need more information about other usages, such as tissues and bacteria (gram positive), please do not hesitate to contact our Technical Assistant Team. The below protocol shows the experimental processes by using cell number (1 ~ 2 x 10^6 cells).
- Common equipment and reagents

  – 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)
  – TE buffer (10 mM Tris-HCl, 0.1 mM EDTA; pH 8.0 - 8.5)

PROTOCOL A - Cell

This method given in the following protocol is suitable for isolation of DNA from 1~2 x 10^6 cells. The protocol can be adapted for DNA isolation from cell number by scaling reagent volume of cell used (see table 1).

1. **Preparation of 1~2 x 10^6 cells into a 1.5 ml tube containing 1 x PBS or cell culture media.**
2. **Harvest pellet of cells by centrifuging at 13,000 ~ 16,000 x g for 5 sec. Remove supernatant leaving behind the cell pellet and remain about 5 ~ 10 μl of the residual liquid.**
   Note : Remain the little volume of solution, it helps cell pellet resuspending. It is not important thing the solution volume in the residual liquid.
   Note : When you use table top centrifuge, 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 Buffer 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°C until the solution is homogeneous.
5. **Add 1.5 μl RNase A Solution to the cell for lysate and incubate at 37°C, for 15~30 minutes.**
   Note : After adding the RNase A at 37°C,it is very efficient by inverting the tube sometimes.
   Note : After the DNA is dissolved in DNA Hydration buffer, add the RNaseA. In general, it is spontaneously removed the RNA when the genomic DNA is extracted in cells.
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 50~150 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 °C for 30 min ~ 60 min or at 4 °C 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 °C or -80 °C. Or store collected DNA at -20 °C.**
15. **Measure the DNA purity O.D 260:280 ratio.**

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PROTOCOL B - Tissue

1. **Take out the target organ from laboratory animal.**
   Note : The fresh animal tissue can be used directly to isolation of genomic DNA. But if the tissues are not used immediately, those should be stored with liquid nitrogen (below -196 ºC) or deep freezer (-80 ºC) for long term preservation
2. **Slice off the prepared sample to suitable size by scalpel or scissor.**
   Note : To reduce disruption and homogenization time, we recommend to slice off it. In case of enzymatic sample lysis, cut the sample 0.6 ~ 1.2 cm (mouse) or 0.3 ~ 0.6 cm (rat) length, then slice the sample into piece as small as possible
3. **Place the sliced sample material into a grinding jar (mortar). Add liquid nitrogen to the mortar and freeze. Keep the sample submerged in liquid nitrogen, and disrupt carefully until the sample is homogenized completely. Allow the liquid nitrogen to evaporate, and proceed immediately to step 4.**
   Note : Disruption and homogenization time depends on the tissue samples. We recommend to be disrupted completely until no tissue clumps are not visible. Clumps of tissue will be difficult to lyse properly and will result in a lower yield of DNA.
4. **Measure 10 ~ 50 mg of ground tissue sample, and transfer to 1.5 ml tube.**
   Note : The repetitive freezing and thawing of frozen tissue will result in the DNA degradation. And more, excess starting amount of tissue sample will result in inefficient lysis, resulting in low DNA yield and purity. If the genomic DNA is prepared from spleen or thymus tissue, no more 10 mg should be used.
5. **Follow the Protocol A (for Cell) from Step 4**

PROTOCOL C - G(-) Bacteria

1. **Pellet the bacteria culture (1~3OD) by centrifugation at 13,000 rpm for 1min, and discard the supernatant. Resuspend the pellet completely with remained supernatant by tapping or vigorous vortexing.**
2. **Follow the Protocol A (for Cell) from Step 4**

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 are slow to rehydrate	• 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.

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EXPERIMENT INFORMATIONS

❖ Table 1 : Solution amounts according to cell number

Table 1 shows the solution volume according to the cell number. For the most suitable efficiency, follow the table.

Cell Number (cells)	Cell Lysis	RNase A	PPT Buffer	Rehyd. Buffer	Yields
100 ~ 10,000	60 µl	0.5 µl	20 µl	25 µl	43 ± 6 ng
10,000 ~ 100,000	300 µl	1.5 µl	100 µl	50 µl	45 ~ 910 ng
1.0 ~ 9.0 ×10^5	600 µl	1.0 µl	200 µl	75 µl	1 ~ 12 mg
1.0 ×10^6	1 ml	5.0 µl	330 µl	100 µl	13 ± 4 mg
1.0 ~ 2.0 ×10^6	2 ml	10 µl	660 µl	200 µl	9 ~ 17 mg
3.0 ~ 5.0 ×10^6	3 ml	15 µl	1.0 ml	300 µ	20 ~ 35 mg
5.0 ×10^6	4 ml	20 µl	1.3 ml	400 µl	35 ± 4 mg
6.0 ~ 9.0 ×10^6	5 ml	25 µl	1.6 ml	500 µl	33 ~ 44 mg
1.0 ×10^7	6 ml	30 µl	2.0 ml	600 µl	48 ± 6 mg
1.0 ~ 2.0 ×10^7	7 ml	35 µl	2.3 ml	700 µl	46 ~ 134 mg
3.0 ~ 5.0 ×10^7	8 ml	40 µl	2.6 ml	800 µl	84 ~ 331 mg
5.0 ×10^7	9 ml	45 µl	3.0 ml	900 µl	328 ± 19 mg
6.0 ~ 9.0 ×10^7	10 ml	50 µl	3.3 ml	1 ml	306 ~ 470 mg
1.0 ~ 2.0 ×10^8	20 ml	55 µl	3.6 ml	2 ml	560 ~ 1,700 mg

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 but calculating the needed DNA concentration.
2) Representative Isopropanol (2-propanol) volume is 90~95% of supernatant which transferred lysate volume. It is good to add Isopropanol (2-propanol) about the 1 volume of supernatant.
3) When the low DNA yield, 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) K562 cells are used in this DNA yield measurement. It depends on cell conditions, experimental conditions, and user.

❖ Table 2 : Useful Numbers for Cell Culture

According to the adherent cell(HeLa cell), it shows about the present cell number.

	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

❖ Table 3 : Tube Selection

According to the cell number, it is easy to select the proper sample tubes.

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

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