Get *pure*DNA Kit-Cell, Tissue *(200 samples)* Technical Manual

Technical Manual (Japanese version) is available at http://www.dojindo.co.jp/manual/gk03.pdf

General Information

Get *pure*DNA Kit-Cell, Tissue enables isolation and purification of genomic DNA from cell cultures and tissue samples in three easy steps: 1) sample lysis, 2) removal of RNA and protein, and 3) DNA recovery using ethanol precipitation. Genomic DNA can be isolated from up to 1 x 10^7 of cells or 30 mg of tissue sample using 1.5 ml tubes. For isolation of genomic DNA from a large number of cells or a large tissue sample, simply increase the volume of each solution. This kit does not require phenol, chloroform, centrifugal columns or filtration tubes. The isolated genomic DNA can be utilized for downstream experiments such as restriction enzyme digestion, ligation, PCR and other enzymatic reactions.

Advantages

- No phenol or chloroform required
- Short DNA isolation time
- No need for spin-colums or filtration tubes
- DNA recovery from a wide range of sample volumes up to 7g of tissue or 2 x 10^9 of cells for 200 samples kit

Kit Contents

- Lysis buffer	110 ml x 1
- Proteinase K solution	1.05 ml x 2
- RNase solution	0.5 ml x 1
- Precipitation solution I	22 ml x 1
- Precipitation solution II	22 ml x 1

Storage Condition

Store at 0-5 °C

- After opening, store Lysis buffer at room temperature.
- When stored the kit at below 20 °C, Lysis buffer may have precipitates at the bottom. If this happens, incubate Lysis buffer at 40-50 °C until the precipitates are dissolved.

Required Equipment and Materials

- 100% ethanol and 70% ethanol
- PBS buffer (for washing cells)
- TE buffer
- 1.5 ml tubes (for small samples)
- 15 ml or 50 ml tubes (for large samples)
- 10 µl, 200 µl and 1000 µl micropipettes and tips
- 20 ml serological pipettes
- water bath
- centrifuge
- vortex mixer
- homogenizer (for tissue samples)

General Protocol - Cell sample -

Please choose a suitable protocol for the number of cells from which genomic DNA is isolated.

For 1 x 10^6 - 3 x 10^6 of cells sample

1) Transfer cell suspension into a 1.5 ml tube, and centrifuge at 1,500 rpm for 5 minutes.

2) Discard the supernatant, and add 500 µl of PBS. Vortex for 5 seconds and centrifuge at 1,500 rpm for 5 minutes.

3) Discard the supernatant, and add 250 µl of Lysis buffer and 10 µl of Proteinase K solution. Dissolve the cells completely by pipetting. Incubate the cell lysate at 65 °C for 10 minutes.
Make sure that all clumps of the cells dissolve before proceeding. This step is essential for high-yield isolation of genomic DNA.

4) Add 2 µl of RNase solution, and vortex for 5 seconds. Leave the solution at room temperature for 2 minutes.

5) Add 50 µl of Precipitation solution I, and vortex for 5 seconds. White precipitate should appear immediately.

6) Add 50 µl of Precipitation solution II, and vortex for 5 seconds. More white precipitate should appear.

7) Centrifuge at 12,000 - 14,000 rpm for 5 minutes and transfer the supernatant to a new 1.5 ml tube.
To avoid contamination, do not disturb the white precipitate during the supernatant transfer. If there are white precipitates remaining in the transferred supernatant, repeat this step.

8) Add an equal volume of ethanol as the supernatant and mix by inverting the tube several times, then vortex for 5 seconds.

9) Centrifuge at 6,000 rpm for 2 minutes. Carefully remove the supernatant without disturbing the precipitate.
A white pellet should be visible on the bottom of the tube. To avoid contamination, do not disturb the white precipitate during the removal of supernatant.

10) Add 1 ml of 70% of ethanol, and vortex for 5 seconds. Centrifuge at 6,000 rpm for 2 minutes. and discard the supernatant.
Make sure that a white pellet is on the bottom of the tube. Carefully remove the supernatant as much as possible.

11) Dry the DNA pellet using a vacuum desiccator for 10 minutes. Dissolve the pellet in TE buffer and use for downstream experiments.
The DNA solution can be stored at 4 °C over one year without any degradation.

For < 1 x 10^6 of cells sample

Follow the protocol for 1 x 10^6 - 3 x 10^6 of cells. In Step 8, add 2 µl of 20 mg/ml glycogen solution (not included) prior to adding ethanol. The glycogen solution makes it easier to identify the DNA precipitate.

For 3 x 10^6 - 1 x 10^7 of cells sample

1) Transfer cell suspension into a 1.5 ml tube, and centrifuge at 1,500 rpm for 5 minutes.

2) Discard the supernatant and add 500 µl of PBS. Vortex for 5 seconds and centrifuge at 1,500 rpm for 5 minutes.

3) Discard the supernatant, and add 500 µl of Lysis buffer and 10 µl of Proteinase K solution. Dissolve the cells completely by pipetting. Incubate the cell lysate at 65 °C for 10 minutes.
Make sure that all clumps of the cells dissolve before proceeding. This step is essential for high-yield isolation of genomic DNA.

4) Add 2 ul of RNase solution and vortex for 5 seconds. Leave the solution at room temperature for 2 minutes.

5) Add 100 µl of Precipitation solution I and vortex for 5 seconds. White precipitate should appear immediately.

6) Add 100 µl of Precipitation solution II and vortex for 5 seconds. More white precipitate should appear.

7) Centrifuge at 12,000 - 14,000 rpm for 5 minutes and transfer the supernatant to a new 1.5 ml tube.
To avoid contamination, do not disturb the white precipitate during the supernatant transfer. If there are white precipitates remaining in the transferred supernatant, repeat this step.

8) Add an equal volume of ethanol as the supernatant, and mix by inverting the tube several times, then vortex for 5 seconds.

9) Centrifuge at 6,000 rpm for 2 minutes. Carefully remove the supernatant without disturbing the precipitate.
A white pellet should be visible on the bottom of the tube. To avoid contamination, do not disturb the white precipitate during the removal of supernatant.

10) Add 1 ml of 70% ethanol, and vortex for 5 seconds. Centrifuge at 6,000 rpm for 2 minutes and discard the supernatant.
Make sure that a white pellet is on the bottom of the tube. Carefully remove the supernatant as much as possible.

11) Dry the DNA pellet using a vacuum desiccator for 10 minutes. Dissolve the pellet in TE buffer and use for downstream experiments.
The DNA solution can be stored at 4 ºC over one year without any degradation.

For 1 x 10^7 - 1 x 10^8 of cells sample

1) Transfer cell suspension into a 15 ml tube, and centrifuge at 1,500 rpm for 5 minutes.

2) Discard the supernatant, and add 4 ml of PBS. Vortex for 5 seconds and centrifuge at 1,500 rpm for 5 minutes.

3) Discard the supernatant, and add 5 ml of Lysis buffer and 40 µl of Proteinase K solution. Dissolve the cells completely by pipetting. Incubate the cell lysate at 65 °C for 10 minutes.
Make sure that all clumps of the cells dissolve before proceeding. This step is essential for high-yield isolation of genomic DNA.

4) Add 20 µl of RNase solution, and vortex for 5 seconds. Leave the solution at room temperature for 2 minutes.

5) Add 1 ml of Precipitation solution I and vortex for 5 seconds. White precipitate should appear immediately.

6) Add 1 ml of Precipitation solution II and vortex for 5 seconds. More white precipitate should appear.

7) Centrifuge at 12,000 - 14,000 rpm for 5 minutes and transfer the supernatant to a new 15 ml tube.
To avoid contamination, do not disturb the white precipitate during the supernatant transfer. If there are white precipitates remaining in the transferred supernatant, repeat this step.

8) Add an equal volume of ethanol as the supernatant and mix by inverting the tube several times, then vortex for 5 seconds.

9) Centrifuge at 6,000 rpm for 2 minutes. Carefully remove the supernatant without disturbing the precipitate.
A white pellet should be visible on the bottom of the tube. To avoid contamination, do not disturb the white precipitate during the removal of supernatant.

10) Add 5 ml of 70% ethanol and vortex for 5 seconds. Centrifuge at 6,000 rpm for 2 minutes and discard the supernatant.
Make sure that a white pellet is on the bottom of the tube. Carefully remove the supernatant as much as possible.

11) Dry the DNA pellet using a vacuum desiccator for 10 minutes. Dissolve the pellet in TE buffer and use for downstream experiments.
The DNA solution can be stored at 4 ºC over one year without any degradation.

The following protocol is for 25-30 mg of tissue samples. Please adjust the solution volumes according to the tissue sample weight.

For 25-30 mg of tissue sample

1) Transfer 25-30 mg of tissue sample into a 1.5 ml tube, and add 400 µl of Lysis buffer and 10 µl of Proteinase K solution.

2) Homogenize the tissue sample using a homogenizer, and incubate at 65 ºC for 10 minutes.

- or -

Incubate at 55 ºC for 2-3 hours with occasional vortexing or pipetting (no need for homogenizing).

3) Leave the tube at room temperature for 2 minutes. Add 2 µl of RNase solution and vortex for 5 seconds. Leave the tube at room temperature for 2 minutes.

4) Add 80 µl of Precipitation solution I and vortex for 5 seconds. White precipitate should appear immediately.

5) Add 80 µl of Precipitation solution II and vortex for 5 seconds. More white precipitate should appear. Leave the tube at room temperature for 2 minutes.

6) Centrifuge at 12,000 - 14,000 rpm for 5 minutes and transfer the supernatant to a new 1.5 ml tube.
To avoid contamination, do not disturb the white precipitate during the supernatant transfer. If there are white precipitates remaining in the transferred supernatant, repeat this step.

7) Add an equal volume of ethanol as the supernatant and mix by inverting the tube several times, then vortex for 5 seconds.

8) Centrifuge at 6,000 rpm for 2 minutes. Carefully remove the supernatant without disturbing the precipitate.
Make sure that a white pellet is on the bottom of the tube. Carefully remove the supernatant as much as possible.

9) Add 1 ml of 70% ethanol and vortex for 5 seconds. Centrifuge at 6,000 rpm for 2 minutes and discard the supernatant.
Make sure that a white pellet is on the bottom of the tube. Carefully remove the supernatant as much as possible.

10) Dry the DNA pellet using a vacuum desiccator for 10 minutes. Dissolve the pellet in TE buffer and use for downstream experiments.
The DNA solution can be stored at 4 ºC over one year without any degradation.

For 1 g of tissue sample

1) Transfer 1 g of tissue sample into a 50 ml centrifuge tube, and add 15 ml of Lysis buffer and 250 µl of Proteinase K solution.

2) Homogenize the tissue sample using a homogenizer, and incubate at 65 ºC for 10 minutes.

3) Cool the tube with a water bath for 2 minutes. Add 70 µl of RNase solution and vortex for 5 seconds. Leave the tube at room temperature for 2 minutes.

4) Add 3 ml of Precipitation solution I and vortex for 5 seconds. White precipitate should appear immediately.

5) Add 3 ml of Precipitation solution II and vortex for 5 seconds. More white precipitate should appear. Leave the tube at room temperature for 2 minutes.

6) Centrifuge at 12,000 - 14,000 rpm for 5 minutes and transfer the supernatant to a new 50 ml tube.
To avoid contamination, do not disturb the white precipitate during the supernatant transfer. If there are white precipitates remaining in the transferred supernatant, repeat this step.

7) Add an equal volume of ethanol as the supernatant and mix by inverting the tube several times, then vortex for 5 seconds.

8) Centrifuge at 6,000 rpm for 2 minutes. Carefully remove the supernatant without disturbing the precipitate.
Make sure that a white pellet is on the bottom of the tube. Carefully remove the supernatant as much as possible.

9) Add 20 ml of 70% ethanol and vortex for 5 seconds. Centrifuge at 6,000 rpm for 2 minutes and discard the supernatant.
Make sure that a white pellet is on the bottom of the tube. Carefully remove the supernatant as much as possible.

10) Dry the DNA pellet using a vacuum desiccator for 10 minutes. Dissolve the pellet in TE buffer and use for downstream experiments.
The DNA solution can be stored at 4 ºC over one year without any degradation.

For 0.5-1.0 cm of tail sample

1) Transfer 0.5-1.0 cm of mouse tail into a 1.5 ml tube, and add 400 µl of Lysis buffer and 10 µl of Proteinase K solution.

2) Incubate at 55 ºC for 4 hours with occasional vortexing or pipetting. Since bone and hair do not dissolve, remove them using autoclaved forceps.

Follow the protocol for 25-30 mg of tissue sample, starting at Step 3.

For 10 cm of tail sample

1) Cut 10 cm of rat tail into 0.5 cm pieces and transfer them into a 50 ml tube, and add 15 ml of Lysis buffer and 250 µl of Proteinase K solution.

2) Incubate at 55 ºC for 4 hours with occasional vortexing or pipetting. Since bone and hair do not dissolve, remove them using autoclaved forceps or centrifuge at 6000 rpm for 2 minutes at 4 ºC or at room temperature, then transfer the supernatant to a new 50 ml tube.

Follow the protocol for 1 g of tissue sample, starting at Step 3.

Troubleshooting (Cell sample)

1) No or low DNA recovery
   - Dissolve cells or cell clumps completely with very through pipetting in Step 3.
   - Make sure that DNA pellet is on the bottom of the tube before discarding the supernatant in Step 9 and 10.

2) Difficult to dissolve the cell pellet in Step 3
   - Prior to adding the lysis buffer, vortex the tube for 5 seconds.
   - Make sure that you are using an appropriate protocol for the number of cells from which DNA is isolated.

3) Low purity of the DNA
   - Dissolve the cells completely by pipetting in Step 3.
   - Do not disturb the white precipitate during the supernatant transfer in Step 7.
   - Make sure to add an equal volume of ethanol as the supernatant in Step 8. If the ethanol volume is too large, RNA may precipitate.

4) Degradation of isolated DNA
   - Use fresh cells.
   - Use autoclaved TE buffer for the solubilization of DNA pellet.

Troubleshooting (Tissue sample)

1) No or low DNA recovery
   - Dissolve the tissue sample or tail tissue completely in Step 2.
   - Make sure that DNA pellet is on the bottom of the tube before discarding the supernatant in Step 8 and 9.

2) Difficult to dissolve the tissue sample in Step 2
   - Prior to transferring tissue sample into a tube, mince or cut it into small pieces.
   - Vortex or pipette every 30 minutes during the incubation. If pipetting, use a pipette tip whose point is cut off, and pipette gently.

3) Too much precipitate after centrifugation in Step 6
   - Prior to adding Precipitation solution I, dissolve the tissue completely.
   - Mix the solution throughly by inverting the tube several times after the addition of Precipitation solution I and II.
   - Increase the centrifugation time if 12,000 - 14,000 rpm (10,000 x g) centrifugation is difficult to achieve.

4) Low purity of the DNA
   - Make sure to incubate at room temperature for 2 minutes after adding RNase solution in Step 3
   - Do not disturb the white precipitate during the supernatant transfer in Step 6.
   - Make sure to add an equal volume of ethanol as the supernatant in Step 7. If the ethanol volume is too large, RNA may precipitate.

5) Degradation of isolated DNA
   - Use fresh tissue samples.
   - Use autoclaved TE buffer for the solubilization of DNA pellet.

DNA Recovery

Table 1. DNA recovery and O.D. value

Sample	DNA Recovery	A_{260}/A_{280}
HeLa cell (1 x 10^7 of cells)	80-120 µg	1.7-1.9
HeLa cell (1 x 10^8 of cells)	1-1.5 mg	1.7-1.9
HL60 cell (1 x 10^7 of cells)	40-60 µg	1.7-1.9
HL60 cell (1 x 10^8 of cells)	500-900 µg	1.7-1.9
Mouse liver (25-30 mg)	40-100 µg	1.7-1.9
Mouse brain (25-30 mg)	20-40 µg	1.7-1.9
Mouse kidney (25-30 mg)	50-60 µg	1.7-1.9
Mouse heart (25-30 mg)	20-25 µg	1.7-1.9
Mouse tail (0.5-1 cm)	40-60 µg	1.7-1.9
Rat liver (1 g)	2-2.5 mg	1.7-1.9
Rat brain (1 g)	600-800 µg	1.7-1.9
Rat kidney (1 g)	1.8-2.3 mg	1.7-1.9
Rat heart (0.8-0.9g)	600-800 µg	1.7-1.9
Rat tail (10 cm)	2.5-3.5 mg	1.7-1.9

Related Product

The following related products are available.

Product Name	Product Code
Get *pure*DNA Kit-Agarose	GK01
Get *pure*RNA Kit	GK04

If you need more infomation, please contact Dojindo technical service.

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