Cat. # 9770A

For Research Use

TaKaRa

# DNAiso Reagent (DNA Extraction Reagent)

Product Manual

v202012Da

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## I. Description

This product is designed to facilitate quick and easy extraction of genomic DNA from samples such as plant and animal tissue, cultured cells, bacteria, and yeast. After homogenizing tissue and cells in DNAiso reagent, ethanol is added to the homogenate to precipitate the genomic DNA. Genomic DNA extracted with DNAiso reagent can be used in procedures such as PCR, Southern blotting, and preparation of genomic libraries.

## II. Components

DNAiso 100 ml

* This reagent includes a protein denaturant. Avoid contact with skin, clothing, etc. In the event of eyes or skin exposure, rinse immediately with copious amounts of water and seek medical attention.

## III. Storage

room temperature.

## IV. Materials Required but not Provided

- 100% ethanol
- 75% ethanol
- Chloroform (for plant tissue)
- TE Buffer (pH 8.0)
- 8 mM NaOH (use within 1 month of preparation. Alternatively, dilute a stock solution of 2 - 4 M NaOH solution that has been prepared within the past 6 months to a final concentration of 8 mM NaOH)
- 1 M HEPES solution (for neutralization)

## V. Protocol

### 1. Quantity of Cell or Tissue that can be Treated with 1 ml of DNAiso

| | |
|---|---|
| Adherent cell | 10 $cm^2$ (35 mm) culture dish |
| Non-adherent cell | 1 - 3 x $10^7$ cells |
| Animal tissue | 25 - 50 mg |
| Plant tissue | 50 - 500 mg |
| Yeast, gram-positive bacteria | 1 - 3 x $10^8$ cells |
| Gram-negative bacteria | 1 - 2 x $10^9$ cells |

### 2. Homogenization

To avoid shearing genomic DNA, use a large bore micropipette tip (e.g., tips with 2 - 3 mm cut from the end) when pipetting genomic DNA solutions.

**[ A ] For adherent cell**

1) Discard the culture medium and wash cells with 1X PBS.
2) Add DNAiso reagent at a ratio of 1 ml per 10 $cm^2$ area of culture dish. Shake the plate gently to spread the reagent over the entire surface of the culture dish. Then, detach the cells by pipetting.
3) Collect the cells with a pipette and transfer to a centrifuge tube. Pipette gently until cells are completely disrupted and the solution becomes transparent.
4) Allow to stand at room temperature for 5 min.

**[ B ] For non-adherent cell**

1) Transfer non-adherent cells to centrifuge tubes. Centrifuge at 4 ℃ for 2 min at 8,000*g*. Discard the supernatant.
2) Add 1 ml of DNAiso reagent per 1 - 3 x $10^7$ cells.
3) Pipette gently until cells are completely disrupted and the solution becomes transparent.
4) Allow to stand at room temperature for 5 min.

**[ C ] For animal tissue**

1) Add 1 ml of DNAiso reagent per 20 - 50 mg of animal tissue.
2) Use a homogenizer such as TaKaRa BioMasher Standard (Cat. #9790A or 9791A) to completely disrupt the tissue.
3) Transfer the homogenized solution to a centrifuge tube and allow to stand at room temperature for 5 min.

- For heart, skin, cartilage or other difficult-to-homogenize tissues, perform the method described in steps C1 - C4' below.
  - C1') Place the tissue in a mortar and add liquid nitrogen to freeze, then grind frozen tissue to a powder with a pestle while adding liquid nitrogen. Insufficient grinding will affect the yield and purity of the resulting genomic DNA.
  - C2') Allow excess liquid nitrogen to evaporate and immediately add sufficient DNAiso reagent (see recommended volumes in V-1). Ensure that the tissue powder is completely immersed in DNAiso reagent.
  - C3') Allow to stand until solution reaches room temperature, then grind powdered tissue until the homogenate is completely transparent.
  - C4') Transfer the homogenate to a centrifuge tube and allow to stand at room temperature for 5 min.

- For small quantities (5 - 10 mg) of liver, spleen, brain or other soft tissue, perform the method shown in steps C1"- C3" below.
  - C1 ") Add 1 ml of DNAiso reagent directly to the tissue in a centrifuge tube.
  - C2 ") Pipette repeatedly until the tissue is completely disrupted and the solution becomes transparent.
  - C3 ") Transfer the homogenate to a new centrifuge tube and allow to stand at room temperature for 5 min.

**[ D ] For plant tissue**

1) Place plant tissue in a mortar and add liquid nitrogen to freeze, then grind frozen tissue to a powder with a pestle while adding liquid nitrogen. Insufficient grinding will affect the yield and purity of the resulting genomic DNA.
2) Allow excess liquid nitrogen to evaporate and immediately add sufficient DNAiso reagent (see recommended volumes shown in V-1). Ensure that the tissue powder is completely immersed in DNAiso reagent.
3) Allow to stand until solution reaches room temperature, then grind powdered tissue until the homogenate is transparent.
4) Transfer the homogenate to a centrifuge tube and allow to stand at room temperature for 5 min.

**[ E ] For yeast and gram-positive bacteria**

1) Recover the cells from the liquid culture media by centrifugation.
2) Place the specimen in a mortar and add liquid nitrogen to freeze, then grind the sample to a powder with a pestle while adding liquid nitrogen. Alternately, digest the cell walls of yeast or gram-positive bacteria using a lytic enzyme such as Zymolyase or lysozyme, respectively. Insufficient digestion or mechanical breakdown of the cell walls will affect the yield and purity of the resulting genomic DNA.
3) Add 1 ml of DNAiso reagent per 1 - 3 x $10^8$ cells.
4) Pipette gently until the homogenate is transparent
5) Transfer the homogenate to the centrifuge tube and allow to stand at room temperature for 5 min.

**[ F ] For gram-negative bacteria**

1) Recover the cells from the liquid culture media by centrifugation.
2) Add 1 ml of DNAiso reagent per 1 - 2 x $10^9$ cells.
3) Pipette gently until cells are completely disrupted, the solution becomes transparent.
4) Allow to stand at room temperature for 5 min.

### 3. Extraction of Genomic DNA

1) Centrifuge the homogenate obtained in step 2 at 10,000*g* for 10 min at 4℃. Transfer the supernatant into a new centrifuge tube, taking care to avoid transferring any insoluble material.
   **Note :** for plant tissue, excess chlorophyll can be eliminated using the method described in steps 3(1) - (3) below.
   3(1) Add an equal volume of chloroform. Mix by inverting the centrifuge tube.
   3(2) Allow to stand at room temperature for 10 min.
   3(3) Centrifuge at room temperature for 10 min. at 12,000*g*. Transfer the top (aqueous) layer to a new centrifuge tube.
2) Add ethanol at 0.5 times the volume of DNAiso used in step 2. Mix by inverting the centrifuge tube.
3) After 1 - 3 min, the DNA will appear as insoluble white flocculent material. Collect this material by wrapping it around a pipette tip and transfer it to a new centrifuge tube. If no precipitate is visible, or if it is too dispersed to easily collect with a pipette tip, centrifuge at room temperature for 2 min at 4,000*g* and recover the pellet.

### 4. Washing the Genomic DNA Pellet

1) Add 1 ml of 75% ethanol and gently mix by inverting the centrifuge tube.
2) Centrifuge at 4 ℃ for 5 min at 12,000*g*. Discard the supernatant. Remove as much of the supernatant as possible.

### 5. Dissolving the Genomic DNA

Dry the genomic DNA pellet at room temperature for several seconds to 1 min, then dissolve the pellet in a suitable volume of TE buffer (pH 8.0) or sterile purified water. Completely dissolving large quantities of genomic DNA will be time-consuming.

If insoluble components (such as polysaccharides) from sources such as liver, skin, or plant tissue are present, remove insoluble material by centrifuging for 10 min at 12,000*g*. In some cases, it may be difficult to completely dissolve large quantities of genomic DNA in TE Buffer or sterile purified water. If this is the case, it may be possible to resuspend the genomic DNA in 8 mM NaOH, but when this is done, HEPES solution must be added immediately after resuspension of the pellet in order to neutralize the alkaline solution. Guidelines for the final pH when a specific volume of 1 M HEPES is added to 1 ml of 8 mM NaOH are given below.

| Final pH | 1 M HEPES Solution |
|---|---|
| 7.0 | 32 μl |
| 7.2 | 23 μl |
| 7.5 | 15.9 μl |
| 7.8 | 11.7 μl |
| 8.0 | 10.1 μl |
| 8.2 | 9.3 μl |
| 8.4 | 8.6 μl |

**Note :** 1. Avoid excessive drying of the genomic DNA pellet, as it may make dissolving of genomic DNA difficult. Do not dry the pellet using heat or a under vacuum.
2. Preparing genomic DNA using this product will normally degrade and eliminate most of the RNA in the sample, but depending on the type of sample, some residual RNA may remain. If necessary, treat the genomic DNA solution with RNase to remove residual RNA.

## VI. Flowchart for Genomic DNA Extraction

Cultured cells, animal/plant tissue, or bacteria
↓
Add DNAiso reagent to homogenize
Incubate 5 min at room temperature
↓
(For plant tissue, treat with chloroform to remove excess chlorophy ll)
↓
Centrifuge for 10 min at 10,000*g*, 4℃
↓
Transfer supernatant to a new centrifuge tube
↓
Add 0.5 volume ethanols (relative to the DNAiso reagent volume) and mix by inverting
↓
Collect the white precipitate
↓
Wash the precipitate with 75% ethanol
↓
Centrifuge for 5 min. at 12,000*g*, 4℃
Discard supernatant and collect the precipitate
↓ Dry pellet
Dissolve in TE buffer, pH 8.0 or sterile purified water.

## VII. Determining Concentration and Purity of Genomic DNA

[ Concentration ]

Measure the $A_{260}$ of the obtained sample to determine the DNA concentration.
Formula for calculating DNA concentration :

$$\text{Concentration of DNA } (\mu\text{g/ml}) = A_{260} \times (\text{Dilution Factor}) \times 50$$

[ Purity ]

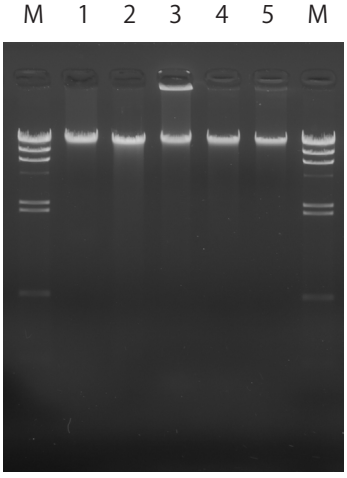
Assess the purity of the genomic DNA by determining the $A_{260}/A_{280}$ ratio. It is desirable for the $A_{260}/A_{280}$ ratio to be within the range of 1.8 to 2.0.

## VIII. Experimental Examples

1. Genomic DNA was extracted from the following samples using 1 ml of DNAiso reagent. The obtained DNA was treated with RNase A, phenol/chloroform extracted, and precipitated with ethanol. DNA yield was determined from the $A_{260}$ value. Each genomic DNA sample (200 ng) was analyzed using 1% agarose gel electrophoresis.

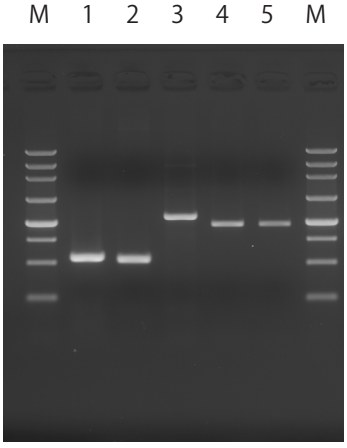
| No. | Material | Amount Used | Yield ( μg)* |
|---|---|---|---|
| 1 | *E. coli* (JM109) | 2 x $10^9$ cells | 11 |
| 2 | Tomato leaf | 500 mg | 10 |
| 3 | Mouse brain | 25 mg | 7 |
| 4 | HeLa cell | 1 x $10^7$ cells | 42 |
| 5 | K562 cell | 1 x $10^7$ cells | 34 |

* Yield after RNA removal




1: *E. coli* (JM109)
2: Tomato leaf
3: Mouse brain
4: HeLa cell
5: K562 cell
M: λ-*Hind* III digest (250 ng)

2. PCR test : PCR was performed using the genomic DNA obtained above as the template with *TaKaRa Ex Taq*® Hot Start Version (Cat. #RR006A). The PCR products were analyzed by electrophoresis on a 1% agarose gel.




| | | amplified gene | |
|---|---|---|---|
| 1. | *E. coli* (JM109) | *araC* | 0.5 kb |
| 2. | Tomato leaf | *Cox I* | 0.5 kb |
| 3. | Mouse brain | *Ccnd2* | 1.2 kb |
| 4. | HeLa cell | *DCARE1a* | 1.0 kb |
| 5. | K562 cell | *DCARE1a* | 1.0 kb |

M: 250 bp DNA Ladder (Dye Plus)

## IX. Troubleshooting

**1. If Yield is Low :**

The yield of genomic DNA will vary depending on the type and amount of the sample. An approximate amount of genomic DNA extracted using DNAiso reagent is shown below.

| Sample | Quantity of Sample | Yield of Genomic DNA |
|---|---|---|
| Mouse Liver | 100 mg | 100 - 400 μg |
| Mouse Kidney | 100 mg | 300- 400 μg |
| Mouse Heart | 100 mg | 200 - 300 μg |
| HL60 cell | 1 x $10^7$ cells | 50 - 70 μg |
| Tomato Leaf | 1 g | 10 - 200 μg |
| *E. coli* | 1 x $10^9$ cells | 3 - 5 μg |

If the yield is markedly lower than expected, consider the following possible causes :

- Homogenization of the sample was incomplete.
- The volume of DNAiso reagent used was insufficient.
- Incomplete dissolving of the genomic DNA precipitate.
- Contamination with DNase occurred during the extraction process.

**2. If the $A_{260}/A_{280}$ Ratio is Low :**

- Addition of too little DNAiso reagent will lead to insufficient protein denaturation. Be sure to use a sufficient amount of DNAiso reagent.
- Incubation for an insufficient length of time after the addition of DNAiso reagent will lead to inadequate dissociation of protein from nucleic acid.
- Ensure that extracted genomic DNA is completely resuspended.

**3. If Extracted Genomic DNA Cannot Be Dissolved :**

- Excessive drying of the pellet following washing with 75% ethanol will make dissolving difficult. Do not dry the pellet excessively. Do not use heat or vacuum to dry the pellet.
- Allow significant time and pipetting to fully dissolve large quantities of extracted genomic DNA.
- If the pellet cannot be dissolved in TE Buffer, pH 8.0 or sterile purified water, try using 8 mM NaOH to resuspend the pellet. Add 1 M HEPES to neutralize after dissolving (see V-5, "Dissolving the Genomic DNA.")

**4. If High Levels of Polysaccharides are Present in the purified Genomic DNA :**

Animal muscle and plant tissues normally contain high levels of polysaccharides. Because it is difficult to remove polysaccharides from extracted genomic DNA, if problems are encountered with your samples, we recommend that you increase the amount of DNAiso reagent used when initially homogenizing the tissue.

**5. If High Levels of Residual RNA are Present in the purified Genomic DNA :**

- More RNA will remain when ethanol precipitation is performed during the extraction of genomic DNA.
- If necessary, treat the genomic DNA solution with RNase.

## X. Related Products

TaKaRa BioMasher Standard (Cat. #9790A/B, 9791A/B)
RNase A (Cat. #740505.50/740505)
*Takara Ex Taq*® Hot Start Version (Cat. #RR006A/B)

## XI. References

Cox R A, *Methods in Enzymology* (Grossmann L and Moldave K., Eds.). (1968) **12** B: 120-129. Academic Press, Orland, FL.