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DNA Isolation Kit for Cells and Tissues

for 10 isolations of 400 mg each for tissue or 5 x 10^7 cultured cells

Cat. No. 11 814 770 001

Principle

The DNA Isolation Kit for Cells and Tissues permits the rapid, large-scale isolation of DNA from cells and tissues. This procedure provides a quick, easy, and safe method for removing contaminating RNA and proteins, resulting in purified genomic DNA ranging in size from 50 – 150 kb.

The procedure includes sample homogenization followed by cellular lysis, in the presence of a strong anionic detergent and Proteinase K. RNA is eliminated with an RNase treatment and proteins are removed by selective precipitation and centrifugation. The purified DNA is subsequently recovered by isopropanol precipitation.

Starting material

- 100 mg – 1 g tissue (research samples)
- 1 x 10^7 to 5 x 10^7 cultured cells (research samples)
- Up to 10^{11} gram negative bacteria
- Up to 3 x 10^5 yeast cells
- 50 – 400 mg mouse tails

Application

- Isolated DNA is suitable for many applications, including standard PCR, long template PCR, and Southern blots.
- DNA isolated can effectively be used with either Taq DNA Polymerase or Expand PCR System products. After quantification we recommend usage of the same amount of DNA per application as typically used in an alternative purification method.

Time required

- Hands on time: 35 min
- Total time for tissue: 2.5 h (plus resuspension time)
- Total time for cultured cells:
  - adherent cells/scraped ≤3.5 h (plus resuspension time)
  - adherent cells/trypsinized ≤4.5 h (plus resuspension time)
  - suspension cells ≤4.0 h (plus resuspension time)

Results

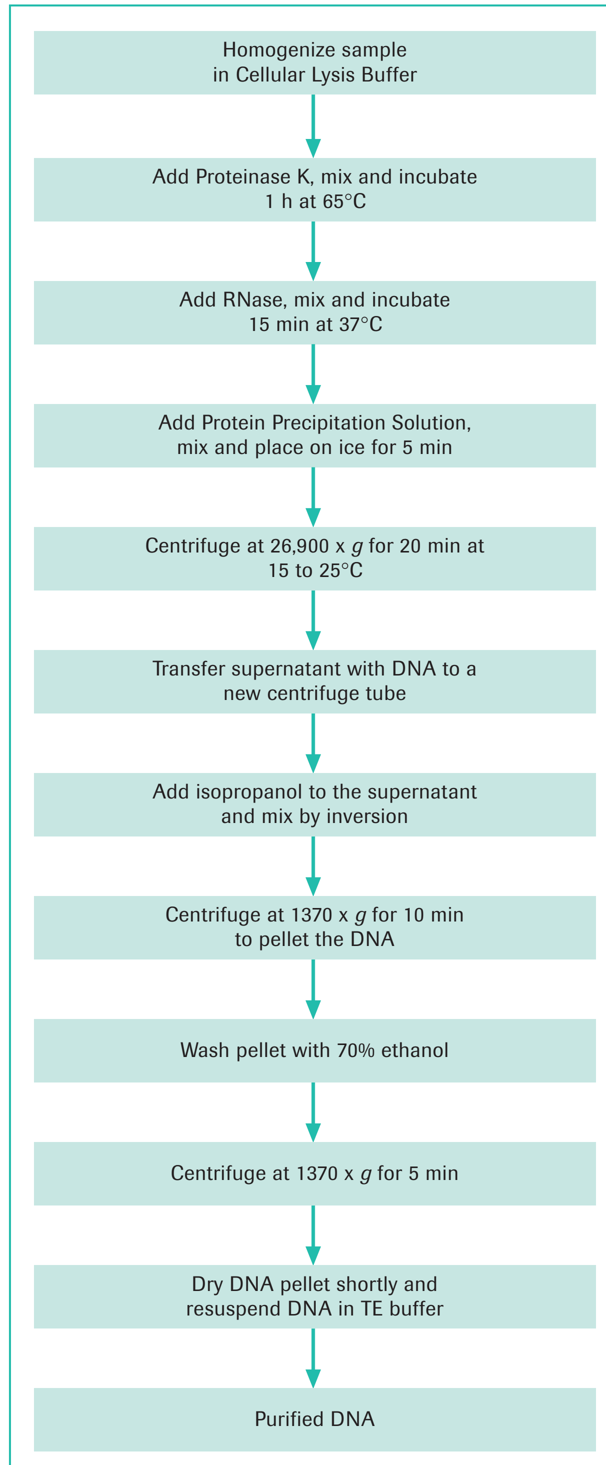
- Yields are determined via spectrophotometry or fluorometry. The $A_{260/280}$ ratio for isolated DNA samples is typically 1.7 – 1.9.
- For detailed data for cells, tissue, bacteria, yeast or mouse tail please refer to the package insert.

Benefits

- **Safe**, eliminates the need for organic extractions or chaotropic reagents.
- **Isolates DNA quickly**, entire procedure can be completed in less than 2.5 hours for tissue (plus resuspension time)
- **Increased DNA recovery**, yield of genomic DNA 2 – 3 times greater than when using column based methods.
- **All reagents necessary** are contained in the kit
- **Flexible**, can be used with a variety of starting material and varying scales

How to use the kit

I. Flow diagram



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II. Kit contents

- Cellular Lysis Buffer, (150 ml)
- Proteinase K Solution (100 µl)
- RNase Solution (5 ml)
- Protein Precipitation Solution (60 ml)

III. Additional materials needed

- 70 % Ethanol
- Isopropanol
- PBS, 1x
- TE Buffer, 1x, pH 8.0 (optional)
- Trypsin (optional)

IV. Procedure for the isolation of DNA from cultured cells

Starting material

- For all procedures, use fresh starting material (tissue, cells from cell culture, bacteria, or yeast) whenever possible.
- If frozen or refrigerated starting material is used, yields may be reduced.

Sample material	General information
Tissue	▶ Store solid tissue at –70°C to avoid degradation of the DNA. ▶ Standard procedure is for a 400 mg sample size. When using less than or greater than 400 mg, follow the alternative procedure, „Optional procedure for DNA isolation from 100 mg – 1 g tissue“.
Cultured cells	▶ Procedure is written for the isolation of DNA from 5 x 10^7 cells. Follow the alternative procedure, „Optional procedure for quantities less than 5 x 10^7 cells“ where appropriate.
Gram Negative Bacteria (*e.g., E. coli*)	▶ Use up to 10^{11} cells and follow the procedure titled, „Isolation of DNA from Gram Negative Bacteria or Yeast“.
Yeast	▶ Count cells and use up to 3 x 10^5 cells. Follow the procedure titled, „Isolation of DNA from Gram Negative Bacteria or Yeast“.
Mouse tail	▶ Use 50 – 400 mg mouse tail and follow the procedure titled, „Isolation of DNA from Mouse Tails“

IVa. Procedure for isolation of DNA from cultured cells

For other sample types see pack insert

Before you begin

- Remove the Cellular Lysis Buffer and the Protein Precipitation Solution from 2 to 8°C prior to starting the procedure.

Prior to use, Resuspend the Cellular Lysis Buffer by placing it at 37°C for approximately 5 min.

- Adjust the waterbaths (65°C, 37°C) and the centrifuge (24°C) to the proper temperatures prior to starting the procedure.

Prior to the isolation, count cells via a Coulter Counter or hemocytometer. The following protocols are optimized for 5 x 10^7 cells. If using fewer cells refer to point IVb.

Pretreatment and harvesting of adherent cells or suspension cell cultures are given in the pack insert.

Lysis and RNA removal

The following procedure applies to 5 x 10^7 cells (adherent or suspension)

1	Homogenize sample until cells are a fine suspension (approximately 10 – 15 s on a medium setting of a Brinkman Polytron Homogenizer or equivalent).
2	Add 10 µl Proteinase K Solution.
3	Vortex sample 2 – 3 s to ensure Proteinase K Solution is mixed with the suspension.
4	Place sample at 65°C for 2 hours.
5	Remove sample from 65°C, loosen cap to vent.
6	Add 500 µl RNase Solution to each sample. *Stock concentration of RNase Solution is 10 mg/ml.*
7	Vortex sample 2 – 3 s to ensure RNase Solution is mixed with the suspension.
8	Place sample at 37°C for 15 min. *The incubation step can be extended up to 1 hour at 37°C if needed.*

Protein precipitation

9	Add 6 ml Protein Precipitation Solution to each sample. Vortex thoroughly 5 – 10 s. *Vortexing is necessary for effective removal of protein from the sample.*
10	Place sample on ice for 5 min. *This step is important because it aids in precipitation of the protein.*
11	Centrifuge the sample at 26,900 x *g* at 15 to 25°C (*e.g.*, 15,000 rpm in a Sorvall RC5B or RC5C). *Ensure that the tube can withstand a centrifugation of 26,900 x g (rmax). If not, transfer sample to another tube at this step (e.g., Nalgene Oak Ridge Centrifuge Tube).* Samples must be centrifuged at 26,900 x *g* (r_{max}) for a minimum of 20 min. Lower centrifuge temperatures or speeds will result in very loose protein pellets, resulting in reduced yields and contamination of the DNA sample with protein.

12. Carefully pipet the supernatant containing the DNA into a new, sterile 50 ml centrifuge tube.

    Keep the pipette away from the protein pellet!

    ! *Pipetting is necessary to avoid white flocculent material at the top of some samples. Pipet from the opposite side of the tube, away from the protein pellet, to ensure that none of the pellet is pipetted into the sample.*

DNA precipitation

13. Add 0.7 volumes of isopropanol to the sample.
14. Invert gently until the upper and lower phases mix.

    ! *Usually DNA "strings" will be visible.*

15. Centrifuge the sample at 1370 x *g* for 10 min (*e.g.*, 2500 rpm in a Sorvall RT6000B or RT7 centrifuge).

    Discard the supernatant.

    ! *Optional Method: Instead of centrifugation, a sterile blunt-ended glass rod may be used to carefully remove the DNA strands from the isopropanol before transferring them to a new sterile tube containing cold 70% ethanol. Swirl until DNA strands are released into the 70% ethanol. Proceed to step 17.*

16. Save the pellet and add 10 ml cold 70% ethanol to the DNA pellet. Dislodge the pellet from the bottom of the tube by tapping the tube.

    ! *This will allow the entire pellet to be washed with the 70% ethanol.*

17. Centrifuge the sample at 1370 x *g* for 5 min (*e.g.*, 2500 rpm in a Sorvall RT6000B or RT7 centrifuge).
18. Discard the supernatant and dry the DNA pellet by placing the sample under vacuum without heat for a few minutes, or until the ethanol is no longer visible.

    or

    Allow the sample to air dry.

    ! *Do not over-dry the DNA pellet as this will make it much more difficult to fully resuspend the DNA.*

19. To resuspend the DNA pellet, add 1 ml TE Buffer, pH 8.0, or desired buffer. Tap tube to dislodge the pellet. Place samples at 50°C for 2 hours to aid resuspension or allow to resuspend at 2 to 8°C overnight.

    ! *If resulting DNA pellet is quite large, more buffer will be needed to resuspend the sample (e.g., 2 – 3 ml).*

20. Store samples at 2 to 8°C until ready to use.

    ! *If desired, samples can be accurately quantified using spectrophotometry or fluorometry.*

IVb. Optional procedure for isolation of DNA from < 5 x 10^7 cultured cells

Modifications

- With slight adjustments to the previous procedure, cell samples from 1 x 10^7 up to 4 x 10^7 can be processed.
- Follow the procedure with the following modification

Cell Number	Cellular Lysis Buffer (ml)	Proteinase K Solution (µl)	RNase Solution (µl)	Protein Precipitation Solution (ml) Calculate the volume of Protein Precipitation Solution by multiplying the total volume x 0.4. See example below.
1 x 10^7	3	2	100	1.2
2 x 10^7	6	4	200	2.5
3 x 10^7	9	6	300	3.7
4 x 10^7	12	8	400	5

Example:
For 1 x 10^7 cells, 3.1 ml (volume of Cellular Lysis Buffer + RNase Solution) x 0.4 = 1.2 ml Protein Precipitation Solution

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Expected DNA yield from different cell lines/cell types

Average yields for adherent and suspension cells

Refer to the following table for average DNA yields from various cell lines.

Cell line/cell type	Average yield per 5 x 10^7 cells	Range per 5 x 10^7 cells
CHOK1/Adherent	977	588 – 1522
COS1/Adherent	2994	1984 – 4182
K562/Suspended	684	463 – 885
PDN-Mouse Hybridoma/ Suspended	1298	1220 – 1487

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V. Troubleshooting the Cells and Tissues DNA Isolation protocol

If you get...	Then, the cause may be...	And you should...
Protein pellet does not form, pellet is soft, or pellet slides from side of tube.	Centrifuge not at 15 to 25°C	▶ Check temperature of centrifuge. Place at 15 to 25°C prior to beginning the procedure.
	Centrifugation speed too low	▶ Samples must be spun at 26,000 x *g* (r_{max}) for 20 min to form protein pellet.
	Incorrect amount of precipitation buffer used	▶ Always calculate the amount of buffer needed for each precipitation.
	Sample not mixed	▶ Mix sample thoroughly by vortexing 10 s.
	Failure to place on ice	▶ Place on ice 5 min prior to centrifugation to aid in precipitation.
	Incubation of sample following addition of Proteinase K Solution exceeded recommended times	▶ Do not exceed recommended times for incubation as this may result in ineffective protein removal from the DNA sample.
DNA does not precipitate	Incorrect amount of isopropanol.	▶ Carefully calculate the amount of isopropanol.
	Sample not mixed completely.	▶ Carefully mix sample by inversion until phases disappear.
Discolored DNA	Certain tissues such as liver may discolor the DNA if the sample is not processed quickly between the isopropanol precipitation and the 70% ethanol wash.	▶ Wash sample with 70% ethanol soon after isopropanol precipitation centrifugation step.
DNA yield lower than expected	Incomplete lysis	▶ Lysis time and buffer volume not correct for sample size.
	Some samples (i.e., muscle, brain, heart) may have low DNA yields due to difficulty in processing the starting material.	▶ Homogenize muscular tissues until completely in suspension.
260/280 ratio too high (>1.9)	RNA contamination	▶ RNase treatment insufficient, increase time up to 1 h.
	Sheared DNA	▶ Do not vortex sample unless stated in procedure.

V. Troubleshooting the Cells and Tissues DNA Isolation protocol, continued

If you get...	Then, the cause may be...	And you should...
260/280 ratio too low (<1.7)	Protein contamination	▶ Increase lysis time and/or amount of Cellular Lysis Buffer. ▶ Do not exceed recommended incubation times as this may result in ineffective protein removal from the DNA sample. ▶ Use pipette for sample removal to prevent protein contamination from the pellet.
	DNA not completely in solution.	▶ Heat DNA to 65°C for 30 min to aid in resuspension.

Typical result with the kit

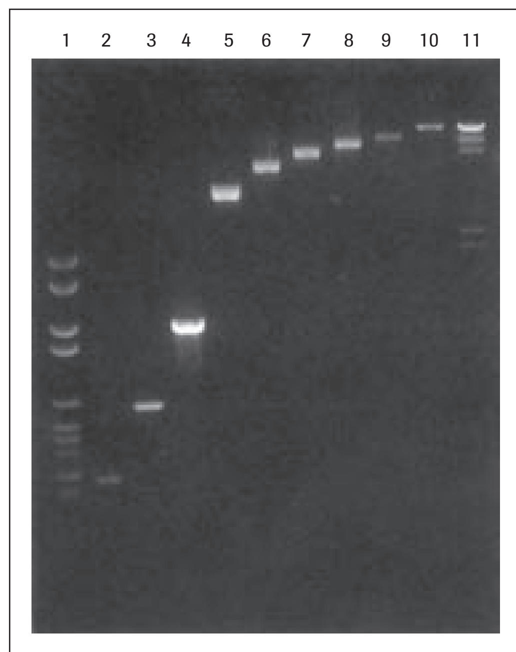

Figure 43: Amplification of 268 bp to 23 kb genomic DNA fragments isolated with the DNA Isolation Kit for Cells and Tissues.

Taq DNA Polymerase, Expand High Fidelity PCR System, and Expand Long Template PCR System were used to amplify fragments isolated with the DNA Isolation Kit for Cells and Tissues.
Lanes 2, 3: Human DMD fragment (268 bp) and mouse c-myc fragment (580 bp) amplified using Taq DNA Polymerase.
Lanes 4, 5, 7 and 8: Human c-myc fragment (1.2 kb), mouse β2 microglobulin fragment (3.6 kb), bovine lysozyme gene fragment (6.9kb), and human tPA gene fragment (9.3 kb) amplified using Expand High Fidelity PCR System.
Lanes 6, 9 and 10: Mouse α-2 collagen gene fragment (5.6 kb and 10.4) and human β-globin fragment (23 kb) amplified using Expand Long Template PCR System.
Lanes 1 and 11: Molecular Weight Markers VI and II.

References

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