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DNA Isolation Kit for Cells and Tissues

Version: 08
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For 10 isolations of DNA from 100 to 1,000 mg mammalian tissue or 5 × 10^7 cultured cells

Cat. No. 11 814 770 001 1 kit
10 isolations

Store the kit at +2 to +8 °C.

1. General Information

1.1. Contents

Vial / Bottle	Label	Content
1	Cellular Lysis Buffer	150 ml
2	Proteinase K, Solution	100 µl
3	RNase, Solution	5 ml
4	Protein Precipitation Solution	60 ml

1.2. Storage and Stability

Storage Conditions (Product)

Kit components are stable at +2 to +8°C until the expiration date printed on the label.

1.3. Additional Equipment and Reagents Required

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

1.4. Application

The DNA Isolation Kit for Cells and Tissues is designed for the simplified and rapid isolation of DNA from cells and tissues, resulting in genomic DNA ranging in size from 50 to 150 kb. The kit eliminates the need for organic extractions, anion exchange columns, or chaotropic reagents used for preparing DNA from cells or tissues.The isolation technique purifies genomic DNA from tissues (up to 1,000 mg), cultured cells (up to 5×10^{7}), gram-negative bacteria (up to 10^{11}), mouse tail, or yeast cells. The procedure removes contaminating RNA and proteins, resulting in high-quality DNA for applications such as standard PCR, long-template PCR, sequencing, and Southern blots. The entire procedure is completed in approximately 2.5 hours, plus resuspension time.

1.5. Preparation Time

Hands on time	▪ 35 minutes
total time for tissue	▪ 2.5 hours (plus resuspension time)
Total time for cultured cells	▪ adherent cells/scraped ≤ 5 hours (plus resuspension time) ▪ suspension cells ≤ 4.0 hours (plus resuspension time) ▪ adherent cells/trypsinized ≤ 4.5 hours (plus resuspension time)

2. How to Use this Product

2.1. Before you Begin

Sample Materials

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

Sample Material	General Information
Tissue	Store solid tissue at −60 to −80°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 to 1000 mg tissue".*
Cultured cells	Procedure is written for the isolation of DNA from 5 × 10^7 cells. For less than that amount, follow the alternative procedure, "Optional procedure for quantities less than 5 × 10^7 cells".
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 × 10^{10} cells. Follow the procedure titled, "Isolation of DNA from Gram--Negative Bacteria or Yeast".

General Considerations

Handling Instructions

- Remove the Cellular Lysis Buffer and the Protein Precipitation Solution from +2 to +8°C environment prior to starting the procedure.
- Adjust the waterbaths (+65°C, +37°C) and the centrifuge (+24°C) to the appropriate temperatures prior to starting the procedure.

⚠ ***Prior to use, resuspend the Cellular Lysis Buffer by placing it at +37°C for approximately 5 min.***
⚠ ***Wear gloves during the assay.***
⚠ ***Do not let buffers touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water; otherwise, the reagent may cause burns. If you spill the reagent, dilute the spill with water before wiping it up.***
⚠ ***Never store or use laboratory materials near human or animal food.***
⚠ ***Always wear gloves and follow standard safety precautions when handling laboratory materials.***

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis / Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease free pipet tips only, to remove aliquots from reagent bottles. Use the general precautions described in the literature.
- Wash hands thoroughly after handling samples and reagents.
- Finish each phase of the PCR/RT-PCR workflow before proceeding to the next phase. For example, you should finish PCR/RT-PCR sample preparation before starting PCR/RT-PCR set-up. Sample preparation, PCR/RT-PCR setup and the PCR/RT-PCR run itself should also be performed in separate locations.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

2.2. Protocols

Procedure for DNA Isolation from 400 mg Tissue

⚠ ***All procedures are usually performed at +15 to +25°C unless otherwise noted.***

Lysis and RNA Removal

1 – For each 400 mg tissue sample to be processed, add 10 ml Cellular Lysis Buffer to a sterile centrifuge tube.
– If using other tissue amounts, see Section “Optional procedure for DNA isolation from 100 to 1000 mg tissue” for modifications to the protocol.

Use a centrifuge tube that will withstand a minimum of 26,900 × g (r_{max}), and accommodate a total volume of 30 ml (Nalgene Oak Ridge Centrifuge Tube, PPCD Cat. No. 3119-0030).

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2 Add 400 mg tissue to each tube.

Tissue may be whole or ground in the presence of liquid nitrogen.

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3 Homogenize sample until tissue is a fine suspension (approximately 10 to 15 seconds), on a medium-setting of a Brinkman Polytron Homogenizer or equivalent.

Whole tissue or muscle tissues may need additional time to finish homogenization.

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4 Add 6.1 µl Proteinase K Solution.

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5 Cap the tube and vortex 2 – 3 seconds to mix the Solution into the suspension.

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6 Place sample at +65°C for 1 hour.

⚠ ***Do not exceed 2 hours as this will affect downstream steps and interfere with DNA isolation.***

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7 Remove sample from +65°C, loosen cap to vent.

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8 Add 400 µl RNase Solution to each sample.

Stock concentration of RNase Solution is 10 mg/ml.

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9 Vortex sample 2 – 3 seconds to mix the Solution into the suspension.

Do not allow tube to completely cool as yields may be reduced.

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10 Incubate sample at +37°C for 15 minutes (may be extended to max. 1 hour).

Protein Precipitation

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11 Add 4.2 ml Protein Precipitation Solution to each sample. Vortex thoroughly 5 – 10 seconds to remove proteins from the sample.

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12 Place sample on ice for 5 minutes to help with precipitation of the protein.

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13 Centrifuge the sample at 26,900 × *g* at +15 to +25°C for 20 minutes (*e.g.* 15,000 rpm in a Sorvall RC5B or RC5C).

Upon centrifugation, a pellet, brown or white depending on the sample will be present.
Lower centrifuge temperatures or speeds will result in very loose protein pellets, reduced yields and contamination of the DNA sample with protein.

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14 – 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 a 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

15 Add 0.7 volumes of isopropanol to the sample.

16 – Invert gently until the upper and lower phases mix.
– Usually DNA “strings” will be visible.

17 – Centrifuge sample at 1,370 × *g* for 10 minutes (*e.g.* 2,500 rpm in a Sorvall RT6000B or RT7 centrifuge).
– Discard the supernatant.
Optional Method:
– 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 19.

18 – Add 10 ml cold 70% ethanol to the DNA pellet.
– Dislodge the pellet from the bottom of the tube by gently tapping the tube.

19 – Centrifuge the sample at 1,370 × *g* for 5 minutes (*e.g.* 2,500 rpm in a Sorvall RT6000B or RT7 centrifuge).
– Discard the supernatant.

20 Dry the DNA pellet by placing the sample under vacuum without heat for 5 minutes, or until the ethanol is no longer visible.
Or:
Allow the sample to air dry.

i *Do not over-dry the DNA pellet, this will make it very difficult to fully resuspend the DNA.*

21 – To resuspend the DNA pellet, add 1 ml TE Buffer, pH 8.0, or desired buffer.
– Tap tube to dislodge pellet.
– Place samples at +50°C for 2 hours or leave overnight at +2 to +8°C to go into solution.
– If the resulting DNA pellet is quite large (e.g. spleen or liver tissue), more buffer will be needed to resuspend the sample. In this case, start with 2 ml resuspension buffer.

22 – Store samples at +2 to +8°C until ready to use.
– If desired, samples can be accurately quantified using spectrophotometry or fluorometry.
Total time for 8 samples: < 2.5 hours plus resuspension

Optional Procedure for DNA Isolation from 100 to 1,000 mg Tissue

Modifications

- Tissue samples from 100 to 1000 mg can be processed by making slight adjustments to the previous procedure.
- Adjust the size of the centrifuge tube as necessary to accommodate the volumes (*e.g.* for 100 mg tissue, a 15 ml tube should be used. For 200 mg and up, use a 30 ml tube).
- The volume of Cellular Lysis Buffer for 800 to 1000 mg tissue stays the same due to the volume constraints of the 30 ml centrifuge tube.

Tissue Weight [mg]	Cellular Lysis Buffer [ml]	Proteinase K Solution [µl]	RNase Solution [µl]	Protein Precipitation Solution [ml]
100	2.5	1.5	100	1.1
200	5.0	3.1	200	2.1
300	7.5	4.6	300	3.1
500	12.5	7.6	500	5.2
600	15.0	9.2	600	6.2
700	17.5	10.7	700	7.3
800 – 1,000	19.0	11.6	760	7.6

Procedure for Isolation of DNA from Cultured Cells

⚠ ***Prior to the isolation, count cells with a Coulter Counter or hemocytometer. The following protocols are optimized for 5 × 10^7 cells. If using fewer cells, refer to section "Optional procedure for isolation of DNA from cultured cells (≤ 5 × 10^7 Cells)" for modifications.***

Adherent Cells: Cell harvesting by scraping

1. Remove media from cells.
2. Gently rinse cells with 8 ml cold 1× PBS.
3. Remove PBS and add 3 ml Cellular Lysis Buffer per 1 × 10^7 cells.
4. Immerse monolayer of cells with Cellular Lysis Buffer and scrape cells to bottom of flask.
5. Pipet 5 × 10^7 cells into a sterile 50 ml centrifuge tube.
6. Proceed to section for Lysis and RNA removal.

Adherent Cells: Cell harvesting by trypsinizing

ⓘ *Scraping is the recommended protocol for adherent cells.*

1. Remove media from cells.
2. Rinse cells with 6 ml of 1% trypsin in PBS.
3. Remove buffer and add another 6 ml of 1% trypsin in PBS.
4. Remove 4 ml buffer, leaving 2 ml, and place cells at +37°C for 2 to 3 minutes to aid in removal of cells from the flask.

5. Cap flask tightly and tap flask firmly with palm of hand to dislodge cells from flask.

6. Immediately add 5 ml of cold PBS and rinse flask thoroughly by pipetting buffer over the sides of the flask, collecting cells at the bottom of the flask.

7. Transfer to a sterile 50 ml centrifuge tube.

8. –Recover the cells by centrifuging at 1,500 × *g* (2,600 rpm in Sorvall RT6000B or RT7) for 10 minutes at +2 to +8°C.
–Remove supernatent.

9. Resuspend cell pellet in 10 ml PBS to wash the cells.

10. Recover cells by centrifuging at 1,500 × *g* for 10 minutes at +2 to +8°C.

11. Remove buffer and add 3 ml Cellular Lysis Buffer per 1 × 10^7 cells (*e.g.* for 5 × 10^7 cells, add 15 ml Cellular Lysis Buffer).

12. Proceed to section for Lysis and RNA removal.

Suspension Cells: Cell harvesting

1. Pipet the required amount of tissue culture media for 5 × 10^7 cells into a sterile 50 ml centrifuge tube.

   This may require more than one tube per sample. Combine to one tube in step 4.

2. Recover cells by centrifuging at 200 × *g* for 10 minutes at +15 to +25°C.

3. Remove all of the media except approximately 1 ml. Gently resuspend cells in the remaining media with a pipette.

4. Add 10 ml 1× PBS total per sample and mix gently.

   If using more than one tube per sample, split the 10 ml equally and combine the sample into one tube.

5. Recover cells by centrifuging at 200 × *g* for 10 minutes at +15 to +25°C.

6. Remove all of the buffer except approximately 1 ml. With a pipette, gently resuspend cells in the remaining media.

7. Add 15 ml Cellular Lysis Buffer per sample of 5 × 10^7 cells.

8. Proceed to section for Lysis and RNA removal.

Lysis and RNA Removal

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

1. Homogenize sample until cells are a fine suspension (approximately 10 to 15 seconds on a medium setting of a Brinkman Polytron Homogenizer or equivalent).

2. Add 10 µl Proteinase K Solution.

3. Vortex sample 2 –3 seconds to mix Proteinase K Solution well into 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 seconds to mix RNase Solution well into the suspension.

8 – Place sample at +37°C for 15 -minutes.
– The incubation step can be extended to 1 hour at +37°C.

Protein Precipitation

9 – Add 6 ml Protein Precipitation Solution to each sample.
– Vortex thoroughly 5 to 10 seconds.

Vortexing is necessary for effective removal of protein from the sample.

10 Place sample on ice for 5 minutes.

This aids in precipitation of the protein.

11 Centrifuge the sample at 26,900 × *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 × g (r_{max}). If not, transfer sample to another tube at this time (e.g. Nalgene Oak Ridge Centrifuge Tube, PPCD Cat. No. 3119-030).

Samples must be centrifuged at 26,900 × g (r_{max}) for a minimum of 20 minutes. Lower centrifuge temperatures or speeds will result in very loose protein pellets, 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 a 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 1,370 × *g* for 10 minutes (*e.g.* 2,500 rpm in a Sorvall RT6000B or RT7 centrifuge).
– Discard the supernatant.
Optional Method:
– 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.

17 Centrifuge the sample at 1,370 × *g* for 5 minutes (*e.g.* 2,500 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 min, 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 more difficult to fully resuspend the DNA.

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19 – To resuspend the DNA pellet, add 1 ml TE Buffer, pH 8.0 or desired buffer.
– Tap tube to dislodge pellet if necessary.
– Place samples at +50°C for 2 hours to aid in 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 to 3 ml).

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20 – Store samples at +2 to +8°C until ready to use.
– Samples can be accurately quantified using spectrophotometry or fluorometry.

Total time for 8 samples:
Scraping: < 3.5 hours plus resuspension
Trypsin: < 4.5 hours plus resuspension
Suspension: < 4.0 hours plus resuspension

Optional Procedure for Isolation of DNA from Cultured Cells (5 × 10^7 Cells)

With slight adjustments to the previous procedure, 1 × 10^7 up to 4 × 10^7 cells can be processed.
Follow the procedure described in section “Procedure for Isolation of DNA from Cultured Cells” with the following modifications:

Cell Number	Cellular Lysis Buffer [ml]	Proteinase K Solution [µl]	RNase Solution [µl]	Protein Precipitation Solution [ml] *)
1 × 10^7	3	2	100	1.2
2 × 10^7	6	4	200	2.5
3 × 10^7	9	6	300	3.7
4 × 10^7	12	8	400	5

*) Calculate the volume of Protein Precipitation Solution by multiplying the total volume × 0.4.
Example: For 1 × 10^7 cells, 3.1 ml (volume of Cellular Lysis Buffer + RNase Solution) × 0.4 = 1.2 ml Protein Precipitation Solution

Procedure for Isolation of DNA from Gram-negative Bacteria or Yeast

Sample Preparation

1. Pipet the required amount of cell culture media for 1 × 10^{11} cells (bacteria) or 3.5 × 10^{10} cells (yeast) into a sterile centrifuge tube.

   This may require more than one tube per sample. Combine to one tube in step 4.
   Total DNA will be isolated from bacteria.
   Bacterial cell count may be quantified by spectrophotometric readings. Yeast must be counted by use of a hemocytometer for accurate results.

2. Recover cells by centrifuging at 200 × *g* for 10 minutes at +15 to +25°C.

3. Remove all of the media except approximately 1 ml. Gently resuspend cells in the remaining media with a pipette.

4. Add 10 ml 1× PBS total per sample, and mix gently.

   If using more than one tube per sample, split the 10 ml equally and combine the sample to one tube.

5. Recover cells by centrifuging at 200 × *g* for 10 minutes at +15 to +25°C.

6. – Remove all of the buffer except approximately 1 ml. Gently resuspend cells in the remaining media with a pipette.
   – Add 15 ml Cellular Lysis Buffer per sample of 1 × 10^{11} cells (bacteria) or 3.5 × 10^{10} cells (yeast).
   Lysis and RNA Removal

7. Homogenize sample until cells are a fine suspension (approximately 10 to 15 seconds on a medium setting of a Brinkman Polytron Homogenizer or equivalent).

8. Add 10 µl Proteinase K Solution.

9. Vortex sample 2 to 3 seconds to ensure Proteinase K Solution is mixed into the suspension.

10. Place sample at +65°C for 2 hours.

11. Remove sample from +65°C, loosen cap to vent.

12. Add 500 µl RNase Solution to each sample.

    Stock concentration of RNase Solution is 10 mg/ml.

13. Vortex sample 2 to 3 seconds to ensure RNase Solution is mixed into the suspension.

14. – Place sample at +37°C for 15 minutes.
    – The incubation step can be extended up to 1 hour at +37°C if needed.

    Protein Precipitation

15. Add 6 ml Protein Precipitation Solution to each sample. Vortex thoroughly (5 – 10 seconds).

    Vortexing is necessary for effective removal of protein from the sample.

16. Place sample on ice for 5 minutes.

    This aids in precipitation of the protein.

17 Centrifuge the sample at 26,900 × *g* at +15 to +25°C for 20 minutes (*e.g.* 15,000 rpm in a Sorvall RC5B or RC5C).

Ensure tube can withstand a centrifugation of 26,900 × g. If not, transfer sample to another tube (Nalgene Oak Ridge Centrifuge Tube, PPCD Cat. No. 3119-030).

Samples must be centrifuged at 26,900 × g (r_{max}) for a minimum of 20 minutes. Lower centrifuge temperatures or speeds will result in very loose protein pellets, reduced yields and contamination of the DNA sample with protein.

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18 Carefully pipet 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 a 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

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19 Add 0.7 volumes of isopropanol to the sample.

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20 – Invert gently until the upper and lower phases mix.
– Usually DNA "strings" will be visible.

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21 – Centrifuge the sample at 1,370 × *g* for 10 minutes (*e.g.* 2,500 rpm in a Sorvall RT6000B or RT7 centrifuge).
– Discard the supernatant.
Optional Method:
– 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 24.

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22 – 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 70% ethanol.

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23 Centrifuge the sample at 1,370 × *g* for 5 minutes (*e.g.* 2,500 rpm in a Sorvall RT6000B or RT7 centrifuge).

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24 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.

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25 – To resuspend the DNA pellet, add 1 ml TE Buffer, pH 8.0, or desired buffer. If necessary, tap tube to dislodge pellet.
– Place samples at +50°C for 2 hours to aid in resuspension or place 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).

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

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

Total time for 8 samples: < 5 hours plus resuspension

Procedure for Isolation of DNA from Mouse Tails

Lysis and RNA Removal

1 Weigh tail section

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2 Grind tissue with mortar and pestle under a thin layer of liquid nitrogen and place into a sterile centrifuge tube.

Alternatively cut tail section into small pieces.
Use a centrifuge tube that will withstand a minimum of 26,900 × g and accommodate a total volume of 30 ml (Nalgene Oak Ridge -Centrifuge Tube, PPCD Cat. No. 3119-0030).
If you follow the alternative procedure, the yield will be reduced.

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3 – For 50 to 100 mg tissue, add 3 ml Cellular Lysis Buffer.
– For 101 to 200 mg tissue, add 5 ml Cellular Lysis Buffer.
– For 201 to 400 mg tissue, add 10 ml Cellular Lysis Buffer.

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4 Add 10 µl Proteinase K Solution.

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5 Cap tube and vortex sample 2 – 3 seconds to ensure Proteinase K Solution is mixed into the suspension.

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6 Place sample at +65°C overnight.

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7 Remove sample from +65°C, loosen cap to vent.

If you cut the tail section into small pieces, they will not completely lyse and will therefore still be present in the solution.

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8 – For 50 to 100 mg tissue, add 120 µl RNase Solution.
– For 101 to 200 mg tissue, add 200 µl RNase Solution.
– For 201 to 400 mg tissue, add 400 µl RNase Solution.

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9 Vortex sample 2 to 3 seconds to ensure RNase Solution is mixed into the sample.

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10 Place sample at +37°C for 15 minutes.

The incubation step can be extended to 1 hour at +37°C.

Protein Precipitation

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11 – For 50 to 100 mg tissue, add 1.2 ml Protein Precipitation Solution.
– For 101 to 200 mg tissue, add 2.1 ml Protein Precipitation Solution.
– For 201 to 400 mg tissue, add 4.2 ml Protein Precipitation Solution.

Ensure tube can withstand a centrifugation of 26,900 × g (r_{max}). If not, transfer sample to another tube (Nalgene Oak Ridge -Centrifuge Tube, PPCD Cat. No. 3119-0030).

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12 Vortex thoroughly 5 to 10 seconds.

This removes protein from the sample.

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13 Place sample on ice for 5 minutes to aid in precipitation of the protein.

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14 Centrifuge the sample at 26,900 × *g* at +15 to +25°C for 20 minutes (*e.g.* 15,000 rpm in a -Sorvall RC5B or RC5C).

Samples must be centrifuged at 26,900 × g (r_{max}) for a minimum of 20 min. Lower centrifuge temperatures or speeds will result in very loose protein pellets, reduced yields and contamination of the DNA sample with protein.

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15 Carefully pipet 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 a 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

16 Add 0.7 volumes of isopropanol to the sample.

17 – Invert gently until the upper and lower phases mix.
– Usually DNA "strings" will be visible.

18 – Centrifuge the sample at 1,370 × *g* for 10 minutes (*e.g.* 2,500 rpm in a Sorvall RT6000B or RT7 centrifuge).
– Discard the supernatant.
Optional Method:
– 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 20.

19 – 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.
– Centrifuge the sample at 1,370 × *g* for 5 minutes (*e.g.* 2,500 rpm in a Sorvall RT6000B or RT7 centrifuge).
– Discard the supernatant.

20 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.

21 – To resuspend the DNA pellet, add 500 µl TE Buffer, pH 8.0, or desired buffer. If necessary, tap tube to dislodge pellet.
– Place samples at +50°C for 2 hours to aid in resuspension, or place at +2 to +8°C overnight.

If resulting DNA pellet is quite large, more buffer will be needed to resuspend the sample (e.g. 1 ml).

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

Samples can be accurately quantified using spectrophotometry or fluorometry.
Total time for 8 samples: < 18 hours plus resuspension

3. Results

Yield

Yields are determined by spectrophotometry or fluorometry. The $A_{260/280}$ ratio for isolated DNA samples is typically 1.7 to 1.9.

Average Yields from 400 mg Tissue

Refer to the following table for average DNA yields from the tissue of various species.
Average yields are scaleable when using less or more than 400 mg.

Species / Tissue	Average yield [µg]	Yield range [µg]
Rat heart	384	357 – 412
Rat brain	427	380 – 448
Rat kidney	1647	1498 – 1746
Rat liver	1276	1138 – 1520
Rat lung	1979	1660 – 2198
Rat spleen	5376	5070 – 5526
Mouse heart	547	502 – 578
Mouse brain	652	623 – 667
Mouse liver	1416	1236 – 1482
Mouse lung	1406	1256 – 1540
Mouse spleen	6861	6213 – 7200
Rabbit brain	342	216 – 558
Rabbit lung	1753	1512 – 1916
Bovine liver	1117	1078 – 1161
Bovine heart	109	78 – 145
Bovine spleen	2899	2510 – 3370
Bovine lung	1153	998 – 1324
Canine spleen	2433	1624 – 2846
Canine kidney	1328	1188 – 1598
Canine liver	1201	1032 – 1328
Porcine spleen	1941	1752 – 2620
Porcine heart	303	289 – 323
Porcine liver	1183	1124 – 1224
Porcine kidney	1252	1182 – 1308
Sheep brain	204	132 – 300
Sheep liver	665	598 – 724

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 5×10^7 cells [µg]	Range per 5×10^7 cells [µg]
CHOK1 / Adherent	977	588 – 1522
COS1 / Adherent	2994	1984 – 4182
K562 / Suspended	684	463 – 885
PDN-Mouse Hybridoma / Suspended	1298	1220 – 1487

Average Yields for Bacteria

Refer to the following table for average DNA yields from bacteria:

E. coli strain	Average yield per 1×10^{11} cells [µg]	Range per 1×10^{11} cells [µg]
BB / 1	1560	1010 – 1940
C	2230	1850 – 2580
K-12	1601	898 – 2304
MM294	2750	2050 – 4030

Average Yields for Yeast

Refer to the following table for average DNA yields from yeast:

Yeast strain	Average yield per 3.5×10^{10} cells [µg]	Range per 3.5×10^{10} cells [µg]
S. cerevisiae	319	273 – 390

Average Yields for Mouse Tail

Refer to the following table for average DNA yields from mouse tail:

Mouse tail weight [mg]	Average yield [µg]	Range [µg]
50	43	18 – 93
100	133	75 – 167
200	351	272 – 454
400	799	716 – 888

4. Troubleshooting

Observation	Possible cause	Recommendation
Protein pellet does not form, pellet is soft, or pellet slides from side of tube.	Centrifugation was not performed 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 × *g* (*r*max) for 20 minutes 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 seconds.
	Failure to place on ice.	Place on ice 5 minutes 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.
$OD_{260/280}$ ratio too high (> 1.9)	RNA contamination	RNase treatment insufficient, increase time up to 1 hour.
	Sheared DNA	Do not vortex sample unless stated in procedure.
$OD_{260/280}$ 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 including protein from the pellet.
	DNA not completely in solution.	Heat DNA to +65°C for 30 minutes to aid in resuspension.

5. Additional Information on this Product

5.1. Test Principle

How this Product Works

- 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 to 150 kb.
- The entire procedure for tissue can be finished in less than 2.5 hours (plus resuspension time).
- 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 (Miller, SA et al. (1988), Lahiri, DK and Schnabel (1993)).

5.2. References

- Barnes WM - PCR amplification of up to 35-kb DNA with high fidelity and high yield from lambda bacteriophage templates (1994) *Proceedings of the National Academy of Sciences of the United States of America* 6, 2216-2220
- Lahiri DK, Schnabel B - DNA isolation by a rapid method from human blood samples: Effects of MgCl2, EDTA, storage time, and temperature on DNA yield and quality (1993) *Biochemical Genetics* 7, 321-328
- Miller SA, Dykes DD, Polesky HF - A simple salting out procedure for extracting DNA from human nucleated cells (1988) *Nucleic Acids Research* 3, 1215-
- Steinhoff UM, Senft B, Seyfert HM - Lysozyme-encoding bovine cDNAs from neutrophile granulocytes and mammary gland are derived from a different gene than stomach lysozymes (1994) *Gene* 2, 271-276

5.3. Quality Control

The following table describes the quality control on each kit:

Tested for...	
Absence of DNase contamination	▪ Each lot of the DNA Isolation Kit for Cells and Tissues is tested for the absence of DNase contamination. ▪ The Cellular Lysis Buffer, Proteinase K -Solution, RNase Solution, and Protein Precipitation -Solution are each incubated with 1 µg pBR322 DNA for 6 hours at +37°C. ▪ The DNA is then visualized by electrophoresis on an agarose gel and compared to a positive control to determine if any linear or nicked plasmid DNA is visible.
DNA isolation and amplification	▪ Each lot of kits is function-tested to purify DNA from bovine liver, followed by specific amplification of a 6,937 kb bovine lysozyme gene (Steinhoff U.M. et al., 1994) fragment via PCR (Barnes W.M., 1994), with the Expand High -Fidelity PCR System. ▪ The 6,937 kb bovine lysozyme gene product is visualized by electrophoresis on an agarose gel, and two samples are compared with a positive control to determine if the same size amplification product is obtained. An intense, 6,937 kb band is visible.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

Layout changes.
Editorial changes.

6.3. Trademarks

All third party product names and trademarks are the property of their respective owners.

6.4. License Disclaimer

For patent license limitations for individual products please refer to: http://technical-support.roche.com.

6.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

6.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.7. Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.
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