ISOLATE DNA Kits

Product Manual

ISOLATE Plasmid Mini Kit
ISOLATE Genomic DNA Mini Kit
ISOLATE Plant DNA Mini Kit
ISOLATE PCR and Gel Kit

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ISOLATE Plasmid Mini Kit

1. KIT CONTENTS

REAGENT	10 MINIPREPS	50 MINIPREPS	250 MINIPREPS
Resuspension Buffer	2 x 2ml	15ml	75ml
Lysis Buffer P	2 x 2ml	15ml	75ml
Neutralization Buffer	2 x 2ml	20ml	100ml
Wash Buffer AP	6ml	30ml	140ml
Wash Buffer BP	4ml	20ml	80ml
Elution Buffer	2ml	2 x 2ml	15ml
Spin Column P	10	50	5 x 50
Collection Tube	10	50	5 x 50
Elution Tube	10	50	5 x 50
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2. DESCRIPTION

ISOLATE Plasmid Mini Kit is designed for the rapid isolation of plasmid DNA from bacterial lysates. Two separate protocols are provided for high copy and low copy plasmids. The protocol is based on alkaline lysis of bacterial cells, followed by neutralization of the lysate and adsorption of the DNA on to silica membrane. The DNA is then washed with a buffer containing ethanol and eluted using an elution buffer. The purified DNA is suitable for all routine molecular biology applications.

Features

- 15 minute protocol
- Typical A_{260}/A_{280} ratio > 1.8
- Clear, easy to follow instructions

Applications

- Isolation of high copy plasmids
- Isolation of low copy plasmids

3. STORAGE

The ISOLATE Plasmid DNA Kit should be stored dry at room temperature. Under these conditions, the kit is stable for 12 months.

4. SAFETY INFORMATION

Always wear gloves and a suitable lab coat when handling the reagents of this kit. For detailed information, refer to the material data safety sheets (MSDSs) available on our website at www.bioline.com.

5. PRODUCT SPECIFICATIONS

Starting volume using LB broth
0.5 - 5ml high copy bacterial cultures
5 - 10ml low copy bacterial cultures

Typical yield
~13µg from 5ml culture
~25µg from 10ml culture

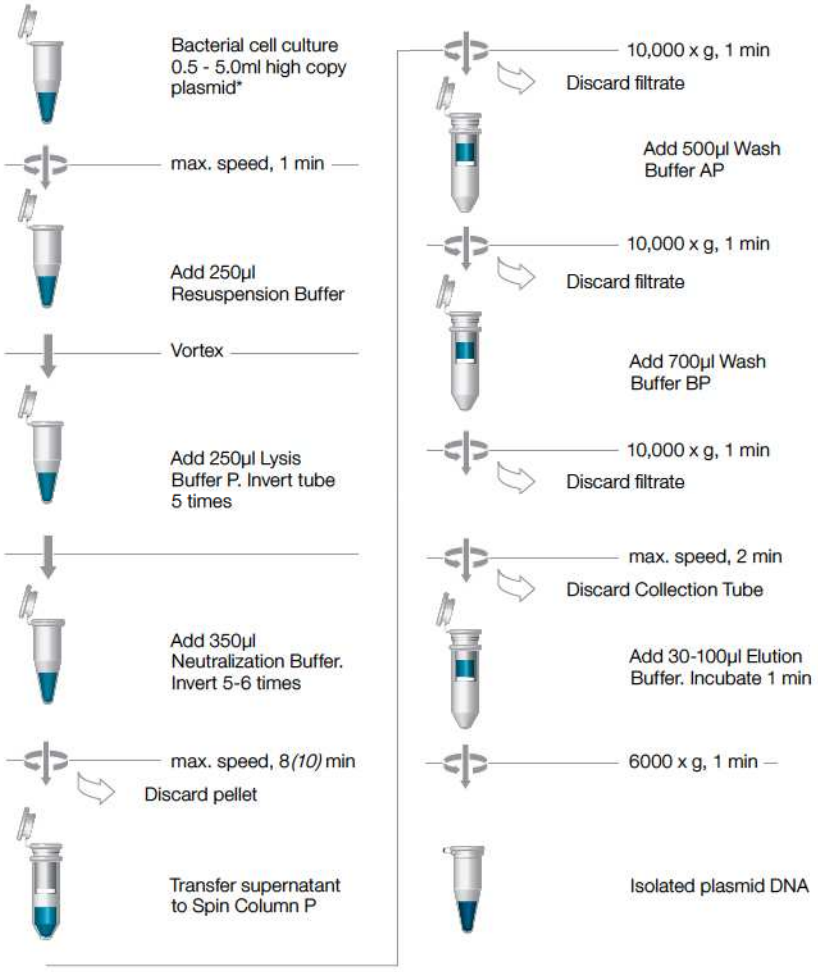
Time required
15 minutes for 5 samples

Typical A_{260}/A_{280} ratio
1.80 – 1.90

Binding capacity
Approximately 25µg

Vector Size
<15kb

Isolation of plasmid DNA



** For low copy number plasmids (5-10ml) double volumes of resuspension lysis and Neutralysing Buffers*

6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

- 1.5ml, 2.0ml or 15ml tubes
- Microcentrifuge with rotor for 1.5ml, 2ml and 15ml tubes
- 96-100% ethanol

7. PROTOCOLS

7.1 Isolation of high copy plasmid DNA

Before you start:

- Before using for the first time, add 96-100% ethanol to Wash Buffer BP as indicated on the bottle and mix.
  Note: All steps to be carried out at room temperature, unless otherwise indicated. Avoid freezing and thawing of starting material.

1. **Transfer 0.5 – 5ml of an overnight bacterial culture to an appropriate tube. Centrifuge for 1 minute at maximum speed to pellet the cells. Remove as much of the supernatant as possible.**
   Note: The presence of the culture medium may inhibit bacterial lysis.

2. **Resuspend the pellet in 250µl Resuspension Buffer. Mix thoroughly by vortexing or pipetting up and down.**
   Note: Ensure that the bacteria are completely resuspended and no cell clumps remain.

3. **Add 250µl Lysis Buffer P and mix carefully by inverting the tube 4-6 times.**
   Note: Mix gently by inverting the tube, do not vortex. Mechanical stresses from vortexing will lead to shearing of genomic DNA which may contaminate the plasmid DNA. Do not allow the lysis reaction to proceed for more than 5 minutes.

4. **Add 350µl Neutralization Buffer to the sample. Mix gently by inverting the tube 4-6 times. Centrifuge for 10 minutes at maximum speed.**

5. **Transfer the sample to Spin Column P placed in a 2ml Collection Tube. Centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and reuse the Collection Tube by placing the Spin Column back in the Collection Tube.**
   Note: Ensure that there is no lysate remaining on the Spin Column. If required, centrifuge the Spin Column again until all liquid has passed through the membrane.

6. **Add 500µl Wash Buffer AP and centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and reuse the Collection Tube by placing the Spin Column back in the Collection Tube.**

7. **Add 700µl Wash Buffer BP and centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and reuse the Collection Tube by placing the Spin Column back in the Collection Tube.**

8. **Centrifuge at maximum speed for 2 minutes to remove all traces of ethanol. Discard the Collection Tube.**

9. **Place Spin Column P into a 1.5ml Elution Tube and add 30-100µl Elution Buffer directly to the Spin Column membrane. Incubate at room temperature for 1 minute. Centrifuge at 10,000 x g (12,000rpm) for 1 minute to elute the plasmid DNA.**
   Note: Use a lower volume of Elution Buffer if a high concentration of DNA is required. Increasing the volume of water will increase the yield but decrease the concentration of DNA. Alternatively, perform two elution steps with an equal volume of Elution Buffer to increase the yield. Use just water if the downstream reactions are EDTA sensitive.

10. **The isolated DNA is ready for use in downstream applications or for storage at -20°C. For long term storage, freeze at -70°C.**

7.2 Isolation of low copy plasmid DNA

Before you start:

- Before using for the first time, add 96-100% ethanol to Wash Buffer BP as indicated on the bottle and mix.
- All steps to be carried out at room temperature, unless indicated otherwise.
  Note: All steps to be carried out at room temperature, unless otherwise indicated. Avoid freezing and thawing of starting material.

1. **Transfer 5 – 10ml of an overnight bacterial culture to 15ml tube. Centrifuge for 1 minute at maximum speed to pellet the cells. Remove as much of the supernatant as possible.**
   Note: The presence of the culture medium may inhibit bacterial lysis.

2. **Resuspend the pellet in 500µl Resuspension Buffer. Mix thoroughly by vortexing or pipetting up and down. Transfer to a 2.0ml tube.**
   Note: Ensure that the bacteria are completely resuspended and no cell clumps remain.

3. **Add 500µl Lysis Buffer P and mix carefully by inverting the tube 6-8 times.**
   Note: Mix gently by inverting the tube, do not vortex. Mechanical stresses from vortexing will lead to shearing of genomic DNA which may contaminate the plasmid DNA. Do not allow the lysis reaction to proceed for more than 5 minutes.

4. **Add 600µl Neutralization Buffer to the sample. Mix gently by inverting the tube 6-8 times. Centrifuge for 10 minutes at maximum speed.**

5. **Transfer 700µl of the sample to Spin Column P placed in a 2ml Collection Tube. Centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and reuse the Collection Tube by placing the Spin Column back on the Collection Tube. Apply the residual sample to the same Spin Column and repeat the centrifugation step.**
   Note: Ensure that there is no lysate remaining on the Spin Column. If required, centrifuge the Spin Column again until all liquid has passed through the membrane.

6. **Add 500µl Wash Buffer AP and centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and reuse the Collection Tube by placing the Spin Column back in the Collection Tube.**

7. **Add 700µl Wash Buffer BP and centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and reuse the Collection Tube by placing the Spin Column back in the Collection Tube.**

8. **Centrifuge at maximum speed for 2 minutes to remove all traces of ethanol. Discard the Collection Tube.**

9. **Place Spin Column P into a 1.5ml Elution Tube and add 50-100µl Elution Buffer directly to the Spin Column membrane. Incubate at room temperature for 3 minute. Centrifuge at 10,000 x g (12,000rpm) for 1 minute to elute the plasmid DNA.**
   Note: Use a lower volume of Elution Buffer if a high concentration of DNA is required. Increasing the volume of water will increase the yield but decrease the concentration of DNA. Alternatively, perform two elution steps with an equal volume of Elution Buffer to increase the yield. Use just water if the downstream reactions are EDTA sensitive.

10. **The isolated DNA is ready for use in downstream applications or for storage at -20°C. For long term storage, freeze at -70°C.**

8. TROUBLESHOOTING GUIDE

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED SOLUTION
Buffer precipitated	Low temperature causing detergent to come out of solution	Re-dissolve by gently warming the solution to 30-40oC for five minutes and shaking gently. Cool to room temperature before using.
Low or no yield	Plasmid did not propagate	Check that optimal growth conditions are met.
	Incomplete lysis	Ensure that no cell clumps are visible after resuspension of pellet with the Resuspension Buffer.
	Bacterial culture too old	For optimal results, do not use cultures grown for longer than 12-16 hours.
	Insufficient amount of antibiotic used in culture medium	Cells without the plasmid of interest may have overgrown cells carrying plasmid of interest.
	Incorrect elution step	Ensure that Elution Buffer is applied directly to the Spin Column membrane.
Poor plasmid quality	Plasmid DNA nicked	Do not exceed the lysis step beyond 5 minutes.
	Genomic DNA contamination	Do not vortex the sample after the lysis step.
Genomic DNA contamination	Spin Column membrane overloaded	The use of rich culture mediums such as Terrific Broth may lead to very high cell densities. In such cases, use smaller starting volumes. The use of Luria Broth to grow cells is recommended.
Plasmid DNA does not perform well in downstream applications	Ethanol carryover during elution	Increase centrifugation time for ethanol removal step.
	Salt carryover during elution	Ensure that Wash Buffers are at room temperature. Check both solutions for salt precipitates. Resuspend any visible precipitate by gentle warming.
	Incorrect Elution Buffer used	If downstream reactions are sensitive to EDTA use water to elute from spin column

ISOLATE Genomic DNA Mini Kit

1. KIT CONTENTS

REAGENT	10 MINIPREPS	50 MINIPREPS	250 MINIPREPS
Lysis Buffer D	5ml	25ml	120ml
Binding Buffer D	2 x 2ml	15ml	70ml
Proteinase K	0.3ml	1.5ml	5 x 1.5ml
Wash Buffer D	6ml	24ml	2 x 60ml
Elution Buffer	2 x 2ml	25ml	110ml
Spin Column D	10	50	5 x 50
Collection Tube	20	2 x 50	10 x 50
Elution Tube	10	50	5 x 50
Product Manual	1	1	1
Bench Protocol Sheet	1	1	1

2. DESCRIPTION

ISOLATE Genomic DNA Mini Kit is designed for the rapid isolation of DNA from a variety of samples. Separate, easy-to-follow protocols are given for DNA isolation from each sample type. The protocol is based on SDS lysis of cells and tissues in the presence of proteinase K, followed by adsorption of the DNA on to silica membrane. The DNA is then washed with a buffer containing ethanol and eluted using an elution buffer. The isolated DNA is suitable for all downstream applications.

Features

- Fast protocol: 15 min after lysis step
- Clear, easy to follow instructions
- High purity DNA: A_{260}/A_{280} – 1.7-2.0

Applications

Isolation of genomic DNA from:

- Fresh or frozen animal tissue
- Paraffin embedded tissue
- Buccal swab
- Eukaryotic cell culture

3. STORAGE

The ISOLATE Genomic DNA Mini Kit should be stored dry at room temperature. Under these conditions, the kit is stable for 12 months. Proteinase K should be stored in aliquots at -20°C after reconstitution (see 7.1), avoid freeze thawing.

4. SAFETY INFORMATION

Always wear gloves and a suitable lab coat when handling the reagents of this kit. For detailed information, refer to the material data safety sheets (MSDSs) available on our website at www.bioline.com.

5. PRODUCT SPECIFICATIONS

Starting material

- Animal tissue (up to 50mg)
- Rodent tail (up to 1cm)
- Paraffin embedded tissue
- Buccal swabs
- Eukaryotic cells (up to 5 x 10^6 cells)

Time required

15 minutes after lysis step

Binding capacity

Approximately 100µg gDNA

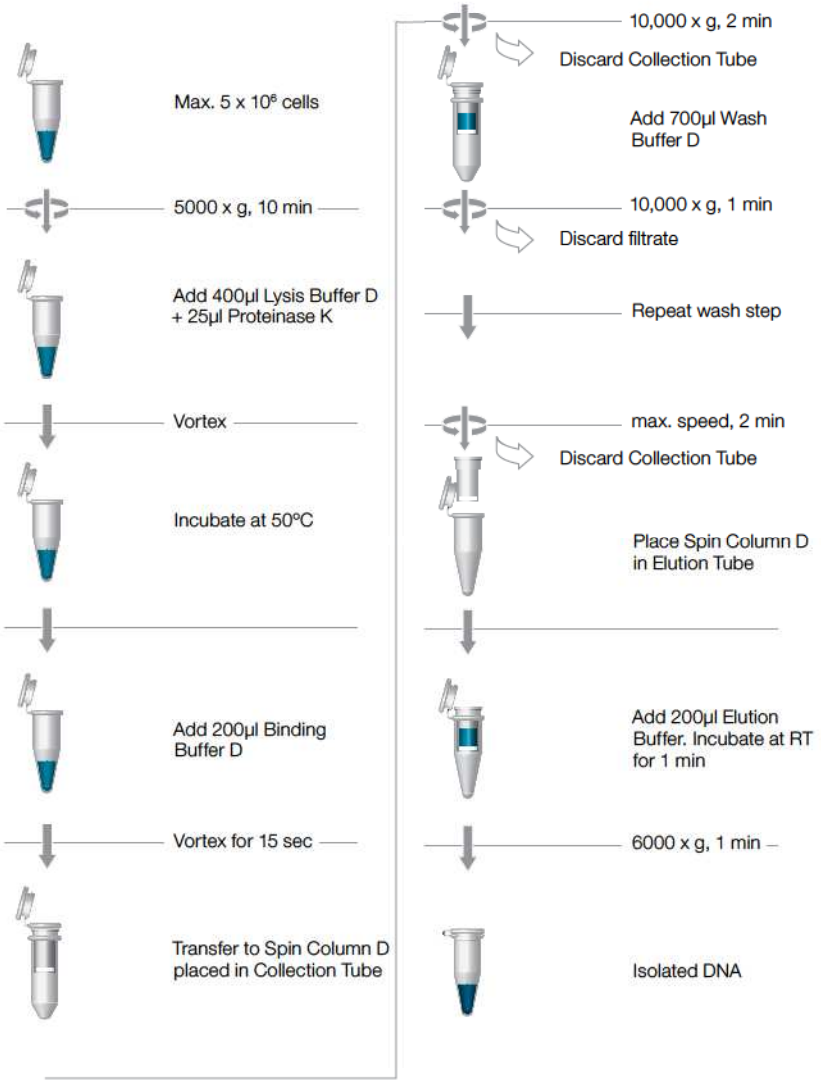
Typical A_{260}/A_{280} ratio

1.7 – 2.0

SAMPLE TYPE AND SIZE	RANGE OF EXPECTED YIELDS (µG)	AVERAGE YIELD (µG)
Buccal swabs, 1 swab	0.2–2	1
Cultured cells, 1–2 x 10^6 cells	5–10	7
Solid animal tissue, 1mg	0.5–10	5
Paraffin-embedded tissue*, 1mg	0.2–2	1

*Yields will depend both on the size and the age of sample processed

DNA isolation from cell culture



6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

- 1.5ml tubes
- Microcentrifuge with rotor for 1.5ml and 2ml tubes
- Shaking water bath
- Xylene or octane (for paraffin embedded tissue)
- RNase A (DNase free) (optional)
- 96-100% ethanol
- ddH_2O

7. PROTOCOLS

7.1 DNA isolation from cell culture

Before you start:

- Add 96-100% ethanol to the Wash Buffers as indicated on the bottles, mix thoroughly.
- Reconstitute lyophilized Proteinase K in water, 0.3ml for the 10 prep kit, 1.5ml for the 50 prep kit and 1.5ml x 5 in the for the 250 prep kit. Aliquot to avoid freeze thawing.
- Prepare a 50°C shaking water bath or heating block for Proteinase K lysis of cell membranes.
  Note: All steps to be carried out at room temperature, unless otherwise indicated. Avoid freezing and thawing of starting material.

1. **Pellet cells (up to 5 x 10^6 cells) by centrifugation for 10 minutes at 5000 x g (7500rpm). Discard supernatant.**

2. **Add 400µl Lysis Buffer D and 25µl Proteinase K and mix well by vortexing vigorously for 5 seconds. Incubate at 50°C until the sample is completely lysed.**
   Note: Vortex the sample intermittently during incubation. Alternately, place on a shaking platform.

3. **Add 200µl Binding Buffer D and mix well by vortexing for 15 seconds.**

4. **Transfer the sample to Spin Column D placed in a 2ml Collection Tube. Centrifuge at 10,000 x g (12,000rpm) for 2 minutes. Discard the Collection Tube and place the Spin Column in a new Collection Tube.**
   Note: Ensure that there is no lysate remaining on the Spin Column. If required, centrifuge the Spin Column again until all liquid has passed through the membrane.

5. **Add 700µl Wash Buffer D. Centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and reuse the Collection Tube.**

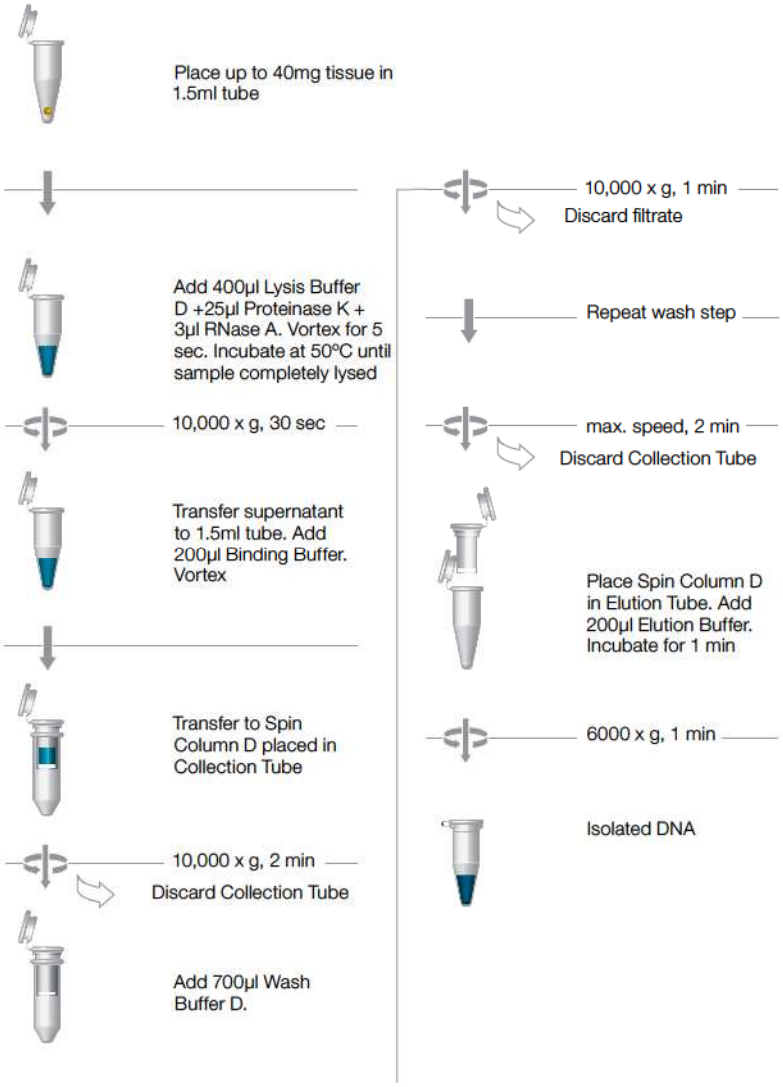
6. **Repeat step 5.**

7. **Centrifuge at maximum speed for 2 minutes to remove all traces of ethanol. Discard the Collection Tube.**

8. **Place Spin Column D in a 1.5ml Elution Tube. Add 200µl Elution Buffer directly to the membrane of the column. Incubate at room temperature for 1 minute. Centrifuge at 6000 x g (8000rpm) for 1 minute.**
   Note: Use a lower volume of Elution Buffer if a high concentration of DNA is required. Increasing the volume of the buffer will increase the yield but decrease the concentration of DNA. Alternatively, a second elution step can be performed to increase the yield of DNA.

9. **The isolated DNA is ready for use in downstream applications or for storage at -20°C. For long term storage, freeze at -70°C.**

DNA isolation from animal tissue



7.2 DNA isolation from animal tissue

Before you start:

- Add 96-100% ethanol to the Wash Buffers as indicated on the bottles, mix thoroughly.
- Reconstitute lyophilized Proteinase K in water, 0.3ml for the 10 prep kit, 1.5ml for the 50 prep kit and 1.5ml x 5 in the for the 250 prep kit. Aliquot to avoid freeze thawing.
- Prepare a 50°C shaking water bath or heating block for Proteinase K lysis of cell membranes.
  Note: All steps to be carried out at room temperature, unless otherwise indicated. Avoid freezing and thawing of starting material.

1. **Cut up to 40mg tissue (up to 1cm rodent tail) into small pieces and place in a 1.5ml microcentrifuge tube.**

2. **Add 400µl Lysis Buffer D and 25µl Proteinase K. Mix by vortexing and incubate at 50°C until the sample is completely lysed. Vortex intermittently to disperse the sample.**
   Note: Complete lysis takes 1 - 3 hours for tissue samples and up to 8 hours for rodent tails. Shaking the sample continuously by placing on a rocking platform will facilitate lysis.

   Optional: If necessary, to remove RNA from the samples, add 4µl RNase A solution (100mg/ml), vortex briefly and incubate for 5 minutes at room temperature.

3. **Centrifuge at 10,000 x g (12,000rpm) for 1 minute. Transfer the supernatant to another 1.5ml microcentrifuge tube.**

4. **Add 200µl Binding Buffer D to the sample. Mix well by vortexing for 15 seconds.**
   Note: Ensure that the sample is homogenized completely.

5. **Transfer the sample to Spin Column D placed in a 2ml Collection Tube. Centrifuge at 10,000 x g (12,000rpm) for 2 minutes. Discard the Collection Tube and place Spin Column D in a new Collection Tube.**
   Note: Ensure that there is no lysate remaining on Spin Column D. If required, centrifuge Spin Column D again until all liquid has passed through the membrane.

6. **Add 700µl Wash Buffer D and centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and reuse the Collection Tube.**

7. **Repeat step 6.**

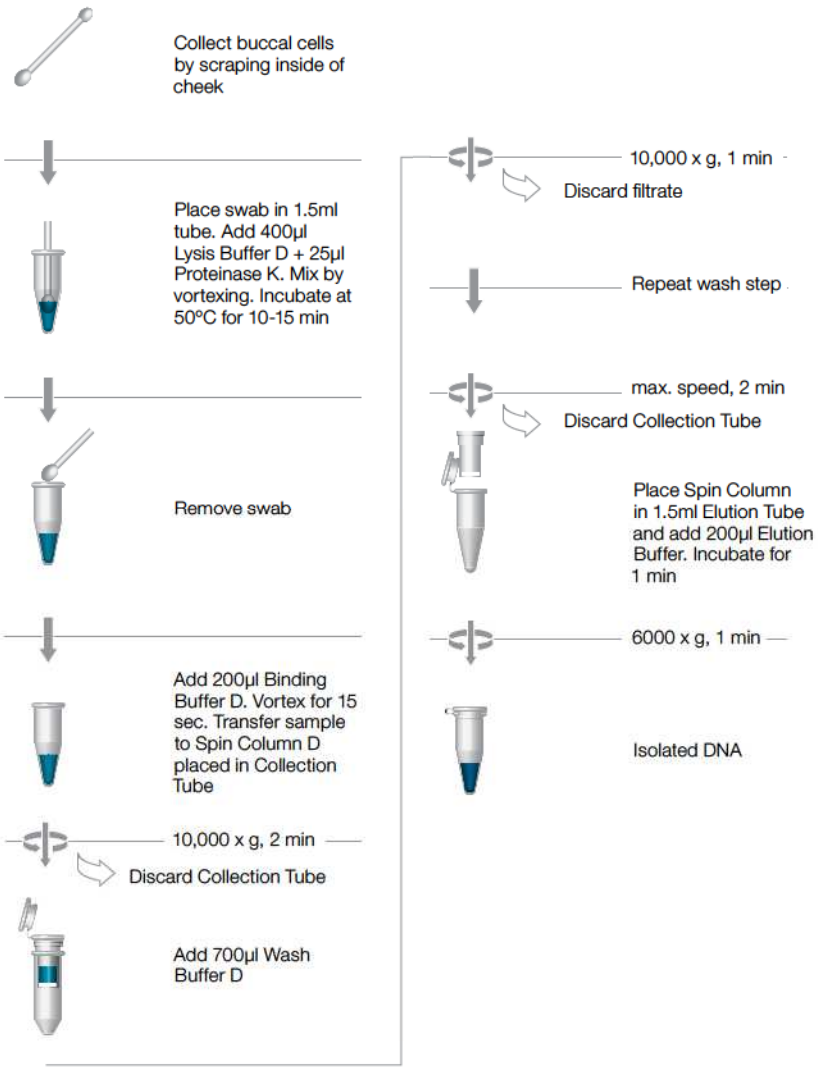
8. **Centrifuge at maximum speed for 2 minutes to remove all traces of ethanol. Discard the Collection Tube.**

9. **Place Spin Column D into a 1.5ml Elution Tube and add 200µl Elution Buffer directly to the Spin Column membrane. Incubate at room temperature for 1 minute. Centrifuge at 6000 x g (8000rpm) for 1 minute to elute the DNA.**
   Note: Use a lower volume of Elution Buffer if a high concentration of DNA is required. Increasing the volume of water will increase the yield but decrease the concentration of DNA. Alternatively, perform two elution steps with an equal volume of Elution Buffer to increase the yield.

10. **The isolated DNA is ready for use in downstream applications or for storage at -20°C. For long term storage, freeze at -70°C.**

7.3 DNA isolation from buccal swab

DNA isolation from buccal swab



Before you start:

- Add 96-100% ethanol to the Wash Buffers as indicated on the bottles, mix thoroughly.
- Reconstitute lyophilized Proteinase K in water, 0.3ml for the 10 prep kit, 1.5ml for the 50 prep kit and 1.5ml x 5 in the for the 250 prep kit. Aliquot to avoid freeze thawing.
- Prepare a 50°C shaking water bath or heating block for Proteinase K lysis of cell membranes.

  Note: All steps to be carried out at room temperature, unless otherwise indicated. Avoid freezing and thawing of starting material.

1. **Collect buccal cells by scraping the inside of the cheek at least 10 times with a buccal swab or brush. Proceed to next step or store the sample by air drying the swab and storing for up to 1 month at room temperature.**

   Note: For best results, the individual should not have consumed food or drink an hour before collection of the sample.

2. **Place the swab in a 1.5ml microcentrifuge tube. Add 400µl Lysis Buffer D and 25µl Proteinase K. Mix by vortexing vigorously for 5 seconds. Incubate at 50°C for 10-15 minutes.**

   Note: Vortex the sample intermittently during incubation. Alternately, place on a shaking platform.

3. **Remove the swab from the tube and scrape it against the side of the tube to recover as much of the liquid as possible. Discard the swab.**

4. **Add 200µl Binding Buffer D to the lysed sample and mix by vortexing for 15 seconds.**

5. **Transfer the sample to Spin Column D placed in a 2ml Collection Tube. Centrifuge at 10,000 x g (12,000rpm) for 2 minutes. Discard the Collection Tube and place the Spin Column in a new Collection Tube.**

6. **Add 700µl Wash Buffer D. Centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and reuse the Collection Tube.**

7. **Repeat step 6.**

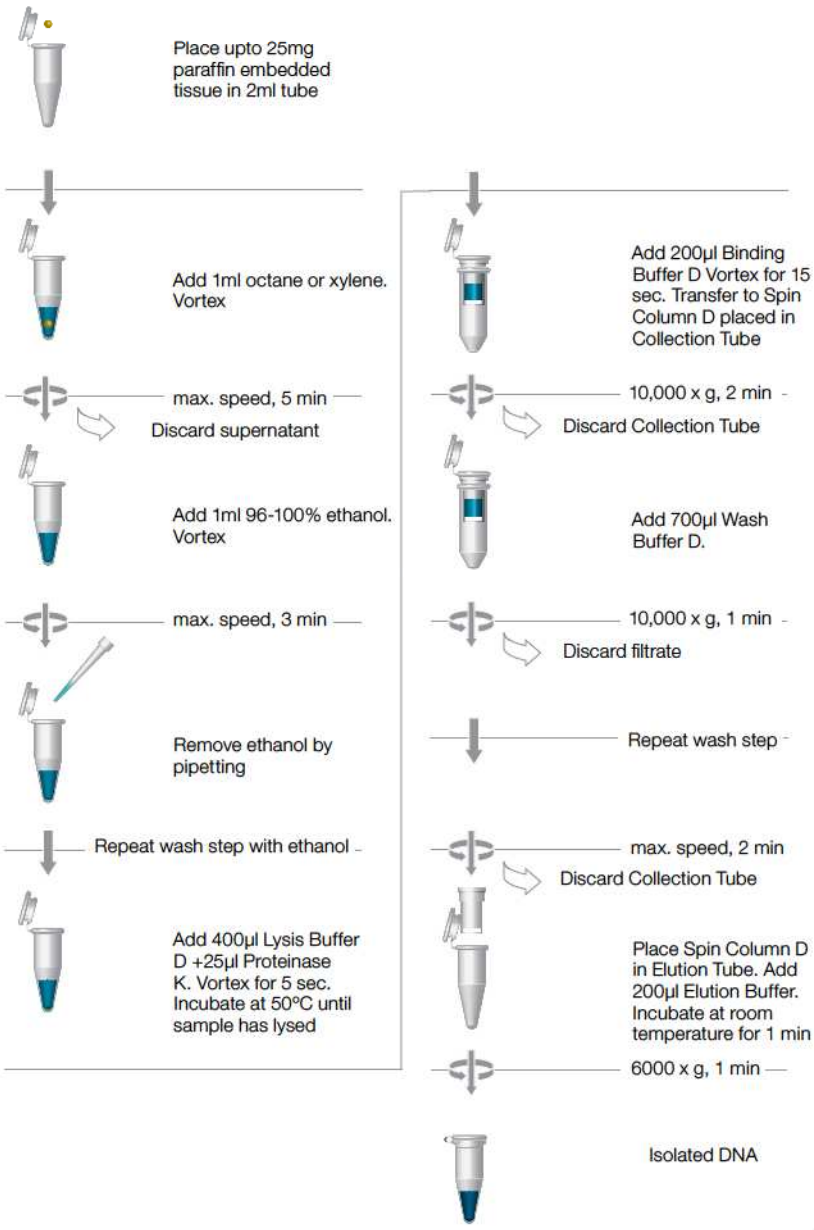
8. **Centrifuge at maximum speed for 2 minutes to remove all traces of ethanol. Discard the Collection Tube.**

9. **Place Spin Column D in a 1.5ml Elution Tube. Add 200µl Elution Buffer directly to the membrane of the column. Incubate at room temperature for 1 minute. Centrifuge at 6000 x g (8000rpm) for 1 minute to elute the DNA.**

   Note: Use a lower volume of Elution Buffer if a high concentration of DNA is required. Increasing the volume of the buffer will increase the yield but decrease the concentration of DNA. Alternatively, a second elution step can be performed to increase the yield of extracted DNA.

10. **The isolated DNA is ready for use in downstream applications or for storage at -20°C. For long term storage, freeze at -70°C.**

DNA isolation from paraffin embedded tissue



7.4 DNA isolation from paraffin embedded tissue

Before you start:

- Add 96-100% ethanol to the Wash Buffers as indicated on the bottles, mix thoroughly.
- Reconstitute lyophilized Proteinase K in water, 0.3ml for the 10 prep kit, 1.5ml for the 50 prep kit and 1.5ml x 5 in the for the 250 prep kit. Aliquot to avoid freeze thawing.
- Prepare a 50ºC shaking water bath or heating block for Proteinase K lysis of cell membranes.

Note: All steps to be carried out at room temperature, unless otherwise indicated. Avoid freezing and thawing of starting material.

1. **Place a small section (under 25mg) of paraffin embedded tissue in a 2ml microcentrifuge tube.**

2. **Add 1ml octane or xylene and vortex carefully to dissolve the paraffin.**
   Note: Continue to vortex until the tissue sample appears transparent.

3. **Centrifuge at maximum speed for 5 minutes at room temperature. Discard the supernatant carefully by aspirating with a pipette WITHOUT DISTURBING THE PELLET.**
   Note: Repeat this step if paraffin is still visible in the sample.

4. **Add 1ml ethanol (96-100%) to the pellet and vortex vigorously.**

5. **Centrifuge at maximum speed for 3 minutes. Remove the ethanol by pipetting.**

6. **Repeat steps 4 and 5.**

7. **Add 400µl Lysis Buffer D and 25µl Proteinase K to the pellet. Mix well by vortexing vigorously for 5 seconds. Incubate at 50ºC until the sample has completely lysed.**

8. **Add 200µl Binding Buffer D and mix by vortexing for 15 seconds.**

9. **Transfer the sample to Spin Column D placed in a 2ml Collection Tube. Centrifuge at 10,000 x g (12,000rpm) for 2 minutes. Discard the Collection Tube and place Spin Column D in a new Collection Tube.**
   Note: Ensure that there is no lysate remaining on the Spin Column. If required, centrifuge the Spin Column again until all liquid has passed through the membrane.

10. **Add 700µl Wash Buffer D. Centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and reuse the Collection Tube.**

11. **Repeat step 10.**

12. **Centrifuge at maximum speed for 2 minutes to remove all traces of ethanol. Discard the Collection Tube. Place the Spin Column in a 1.5ml Elution Tube.**

13. **Add 50-100µl Elution Buffer. Incubate at room temperature for 1 minute. Centrifuge at 6000 x g (8000rpm) for 1 minute to elute the DNA.**
    Note: Use a lower volume of Elution Buffer if a high concentration of DNA is required. Increasing the volume of the buffer will increase the yield but decrease the concentration of DNA. Alternatively, a second elution step can be performed to increase the yield of DNA.

14. **The isolated DNA is ready for use in downstream applications or for storage at -20°C. For long term storage, freeze at -70°C.**

8. TROUBLESHOOTING GUIDE

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED SOLUTION
Clogged Spin Column	Insufficient disruption or homogenization	Reduce starting material.
	Insufficient centrifugation	Increase centrifugation speed and time.
Low DNA yield	Insufficient disruption or homogenization	Reduce amount of starting material.
	Incomplete elution	Incubate sample in Elution Tube with Elution Buffer for up to 5 minutes and repeat elution step.
Low DNA concentration	High elution volume	Elute DNA with a lower volume. Do not use less than 20µl.
DNA degraded or sheared	Inappropriate handling and storing of starting material	Ensure proper handling and storage of samples. Ensure that all steps are followed quickly.
	Poor sample quality	Use fresh samples as far as possible as old samples often yield degraded DNA.
A_{260}/A_{280} ratio too high	RNA contamination	Perform optional RNase treatment.
	Too much starting material	Reduce amount of starting material.
DNA does not perform well in downstream applications	Ethanol carryover during elution	Increase centrifugation time for ethanol removal step.
	Salt carryover during elution	Ensure that Wash Buffers HS and LS are at room temperature. Check both solutions for salt precipitates. Resuspend any visible precipitate by gentle warming.
	Incorrect Elution Buffer used	If downstream reactions are sensitive to EDTA use water to elute from spin column.

ISOLATE Plant DNA Mini Kit

1. KIT CONTENTS

REAGENT	10 MINIPREPS	50 MINIPREPS	250 MINIPREPS
Lysis Buffer PD	5ml	25ml	120ml
Binding Buffer PD	2x2ml	15ml	70ml
Precipitation Buffer	1.5ml	6ml	30ml
Wash Buffer PD	6ml	24ml	2 x 60ml
Elution Buffer	2 x 2ml	25ml	110ml
Spin Column PD1	10	50	5 x 50
Spin Column PD2	10	50	5 x 50
Collection Tube	30	3 x 50	15 x 50
Elution Tube	10	50	5 x 50
Product Manual	1	1	1
Bench Protocol Sheet	1	1	1

2. DESCRIPTION

ISOLATE Plant DNA Mini Kit is designed for the rapid isolation of plant DNA from a variety of plant samples, and replaces cumbersome DNA isolation procedures such as CTAB, phenol, or chloroform extraction. Up to 100mg dry plant material and up to 180mg wet plant material can be processed per Spin Column. The purified DNA is suitable for use with all downstream applications.

The protocol does not require the use of Proteinase K, which means that all components can be conveniently stored at room temperature.

Features

- High purity DNA: A_{260}/A_{280} – 1.7-2.0
- DNA isolated in 30-40 minutes
- Clear, easy to follow instructions

Applications

Isolation of genomic DNA from:

- Fresh plant tissue
- Frozen plant tissue
- Lyophilized plant tissue
- Herbarium specimens

3. STORAGE

The ISOLATE Plant DNA Mini Kit should be stored dry at room temperature. Under these conditions, the kit is stable for 12 months.

4. SAFETY INFORMATION

Always wear gloves and a suitable lab coat when handling the reagents of this kit. For detailed information, refer to the material data safety sheets (MSDSs) available on our website at www.bioline.com.

5. PRODUCT SPECIFICATIONS

Starting material

Fresh, frozen or dried plant material

Time required

30-40 minutes

Binding capacity

Approximately 100µg gDNA

Typical A_{260}/A_{280} ratio

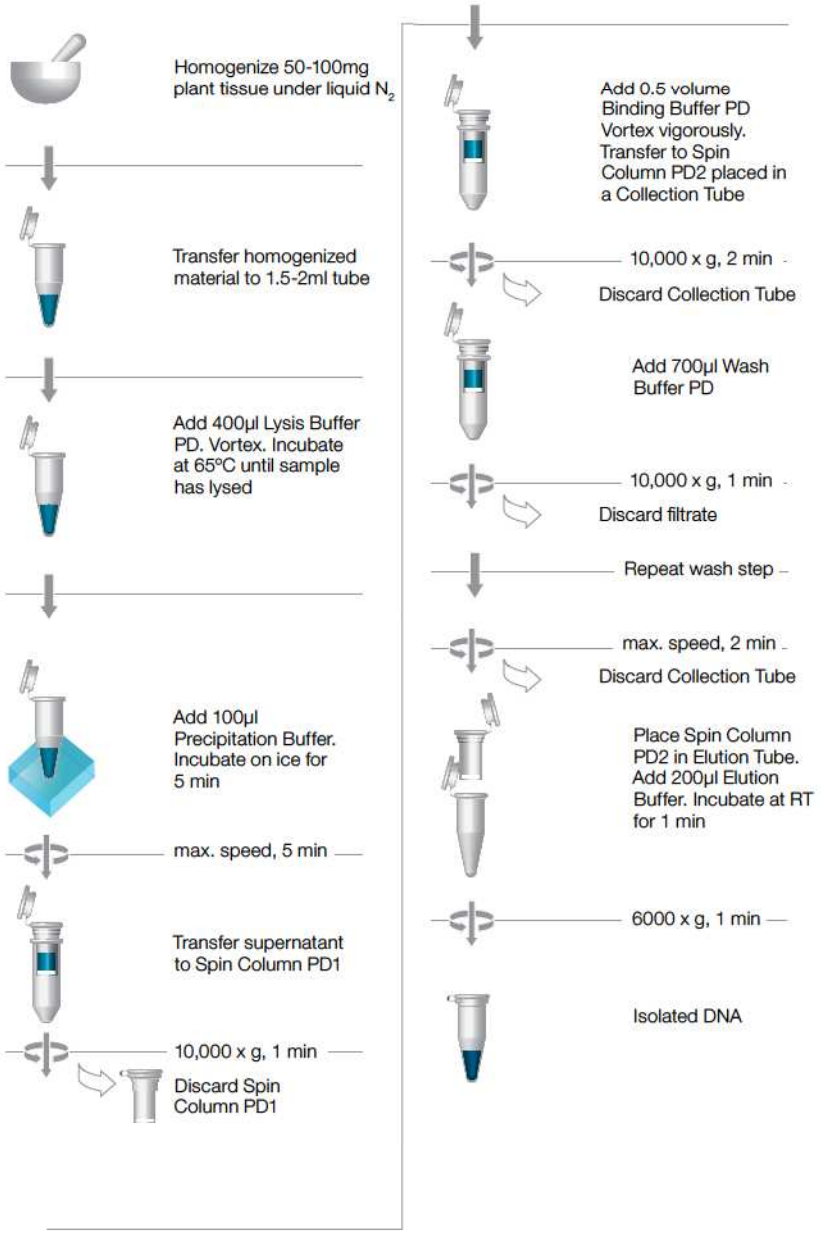
1.8 – 2.0

Typical yield*

3-30µg

**DNA yields vary between different species and tissues depending on genome size, ploidy, cell number, and age of tissue sample.*

DNA isolation from plant tissue



6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

- 1.5ml tubes
- Microcentrifuge with rotor for 1.5ml and 2ml tubes
- Liquid nitrogen or homogenizer
- Ice bucket
- Shaking water bath
- RNase A (DNase free) (optional)
- 96-100% ethanol
- ddH_2O

7. PROTOCOL

7.1 DNA isolation from plant tissue

Before you start:

- Add 96-100% ethanol to the Wash Buffers as indicated on the bottles, mix thoroughly.
- Prepare a 65°C shaking water bath or heating block.
  Note: All steps to be carried out at room temperature, unless otherwise indicated. Avoid freezing and thawing of starting material.

1. **Homogenize 50-100mg plant tissue under liquid nitrogen using a mortar and pestle.**
   Note: If using wet plant tissue, use 120-180mg starting material.

2. **Transfer the homogenized material to a 1.5ml or 2.0ml tube and add 400µl Lysis Buffer PD.**

   Optional: If necessary, to remove RNA from the samples, add 3µl RNase A solution (100mg/ml) and vortex briefly.

3. **Incubate at 65°C until the sample is lysed (approximately 30 minutes). Vortex intermittently to disperse the sample.**
   Note: Lysis may take longer than 30 minutes. Shaking the sample continuously by placing on a rocking platform will facilitate lysis.

4. **Add 100µl Precipitation Buffer and vortex for 5 seconds.**

5. **Incubate on ice for 5 minutes.**

6. **Centrifuge at maximum speed for 5 minutes. Transfer the supernatant to Spin Column PD1 placed in a 2.0ml microcentrifuge tube.**

7. **Centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard Spin Column PD1 and save the filtrate.**

8. **Add 0.5 volume Binding Buffer PD to the filtrate. Mix well by pipetting up and down several times.**
   Note: Ensure that the sample is homogenized completely.

9. **Transfer the sample to Spin Column PD2 placed in a 2ml Collection Tube. Centrifuge at 10,000 x g (12,000rpm) for 2 minutes. Discard the Collection Tube and place the Spin Column in a new Collection Tube.**
   Note: Ensure that there is no lysate remaining on the Spin Column. If required, centrifuge the Spin Column again until all liquid has passed through the membrane.

10. **Add 700µl Wash Buffer PD and centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and reuse the Collection Tube.**

11. **Repeat step 11.**

12. **Centrifuge at maximum speed for 2 minutes to remove all traces of ethanol. Discard the Collection Tube.**

13. **Place Spin Column PD2 into a 1.5ml Elution Tube and add 200µl Elution Buffer directly to the Spin Column membrane. Incubate at room temperature for 1 minute. Centrifuge at 6000 x g (8000rpm) for 1 minute to elute the DNA.**
    Note: Use a lower volume of Elution Buffer if a high concentration of DNA is required. Increasing the volume of water will increase the yield but decrease the concentration of DNA.

14. **The isolated DNA is ready for use in downstream applications or for storage at -20°C. For long term storage, freeze at -70°C.**

8. TROUBLESHOOTING GUIDE

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED SOLUTION
Clogged Spin Column	Insufficient disruption or homogenization	Reduce starting material.
	Insufficient centrifugation	Increase centrifugation speed and time.
Low DNA yield	Insufficient disruption or homogenization	Reduce amount of starting material.
	Incomplete elution	Incubate sample in Elution Tube for up to 5 minutes and repeat elution step.
	Insufficient mixing with Binding Buffer	Mix sample with Binding Buffer by pipetting up and down several times, or vortex sample.
Low DNA concentration	High elution volume	Elute DNA with a lower volume.
DNA degraded or sheared	Inappropriate handling and storing of starting material	Ensure proper handling and storage of samples. Ensure that all steps are followed quickly.
	Poor sample quality	Use fresh samples as far as possible as old samples often yield degraded DNA.
A_{260}/A_{280} ratio too high	RNA contamination	Perform optional RNase treatment.
	Too much starting material	Reduce amount of starting material.
DNA does not perform well in downstream applications	Ethanol carryover during elution	Increase centrifugation time for ethanol removal step
	Salt carryover during elution	Ensure that Wash is at room temperature. Check solutions for salt precipitates. Resuspend any visible precipitate by gentle warming.
	Incorrect Elution Buffer used	If downstream reactions are sensitive to EDTA use water to elute from spin column.

ISOLATE PCR and Gel Kit

1. KIT CONTENTS

REAGENT	10 MINIPREPS	50 MINIPREPS	250 MINIPREPS
Gel Solubilizer	8ml	40ml	200ml
Binding Optimizer	0.7ml	2 x 2ml	18ml
Binding Buffer A	6ml	30ml	140ml
Wash Buffer A	4ml	16ml	2 x 40ml
Elution Buffer	2ml	2 x 2ml	20ml
Spin Column A	10	50	5 x 50
Collection Tube	10	50	5 x 50
Elution Tube	10	50	5 x 50
Product Manual	1	1	1
Bench Protocol Sheet	1	1	1

2. DESCRIPTION

ISOLATE PCR and Gel Kit is designed for the extraction of DNA fragments from agarose gel slices, and for the purification of post-PCR products. It provides spin columns, buffers, and collection tubes for silica-membrane-based purification of DNA fragments of 100bp – 30kb from up to 300 mg gel slices. The spin columns are designed to allow elution in very small volumes (as little as 10µl) delivering highly concentrated DNA in high yields. A fast and easy-to-follow protocol is given for each application. The isolated DNA is suitable for use with all downstream molecular biology applications.

Features

- 3-minute protocol for PCR products purification
- 18 minute protocol for DNA extraction from gels
- Excellent recovery rate

Applications

Isolation of DNA from:

- *In vitro* transcription
- Labeling
- Ligation and transformation
- Microinjection
- PCR
- Restriction digestion
- sequencing

3. STORAGE

The ISOLATE PCR and Gel Kit should be stored dry at room temperature. Under these conditions, the kit is stable for 12 months.

4. SAFETY INFORMATION

Always wear gloves and a suitable lab coat when handling the reagents of this kit. For detailed information, refer to the material data safety sheets (MSDSs) available on our website at www.bioline.com.

5. PRODUCT SPECIFICATIONS

DNA extraction from agarose gel

Starting material
TAE or TBE agarose gels (up to 300mg)

Time required
18 minutes

Rate of recovery
Up to 95%

Lengths recovered
100bp-30Kb

Purification of PCR products

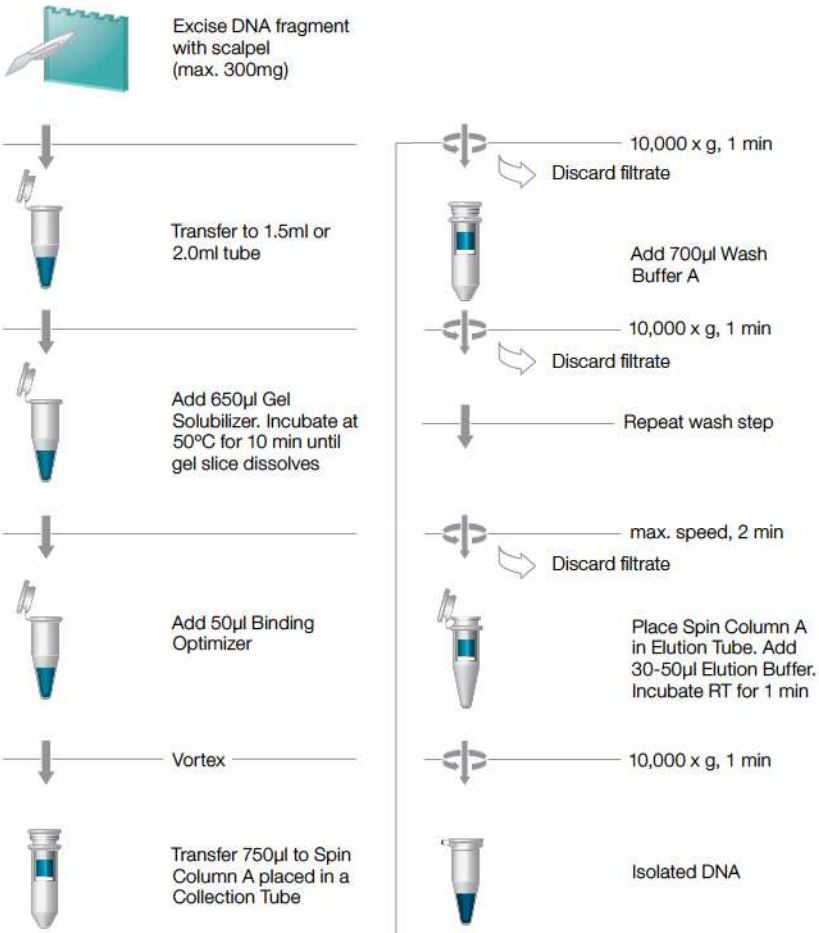
Starting material
PCR mixtures

Time required
3 minutes

Rate of recovery
75-95%

Lengths recovered
100bp-30Kb

DNA extraction from agarose gel



6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

- 1.5ml or 2.0ml tubes
- Microcentrifuge with rotor for 1.5ml tubes
- Shaking water bath
- 96-100% ethanol

7. PROTOCOLS

7.1 DNA extraction from agarose gel

Before you start:

- Add 96-100% ethanol to the Wash Buffers as indicated on the bottles, mix thoroughly.
- Prepare a 50°C shaking water bath or heating block.
- The Elution Buffer can be prewarmed to 50°C before use to increase yield.
  Note: All steps to be carried out at room temperature, unless otherwise indicated. Avoid freezing and thawing of starting material.

1. **Excise the DNA fragment from the agarose gel with a clean, sharp scalpel.**
   Note: Minimize the size of the gel slice. Do not use more than 300mg gel per Spin Column.

2. **Transfer the gel slice to a 1.5ml or 2.0ml tube. Add 650µl Gel Solubilizer.**

3. **Incubate the tube for 10 minutes at 50°C in a water bath until the gel slice has completely dissolved.**
   Note: Use a shaking water bath to help dissolve the gel slice. Alternatively, vortex the sample 3-4 times during incubation.

4. **Add 50µl Binding Optimizer to the sample. Mix by pipetting up and down or by vortexing.**

5. **Transfer 750µl of the sample to Spin Column A placed in a 2ml Collection Tube. Centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and reuse the Collection Tube by placing the Spin Column back in the Collection Tube. Load any residual solution and repeat the centrifugation step. Reuse the Collection Tube.**
   Note: Ensure that there is no lysate remaining on the Spin Column. If required, centrifuge the Spin Column again until all liquid has passed through the membrane.

6. **Add 700µl Wash Buffer A and centrifuge at 10,000 x g (12,000rpm) for 1 minute. Discard the filtrate and reuse the Collection Tube by placing the Spin Column back in the Collection Tube.**

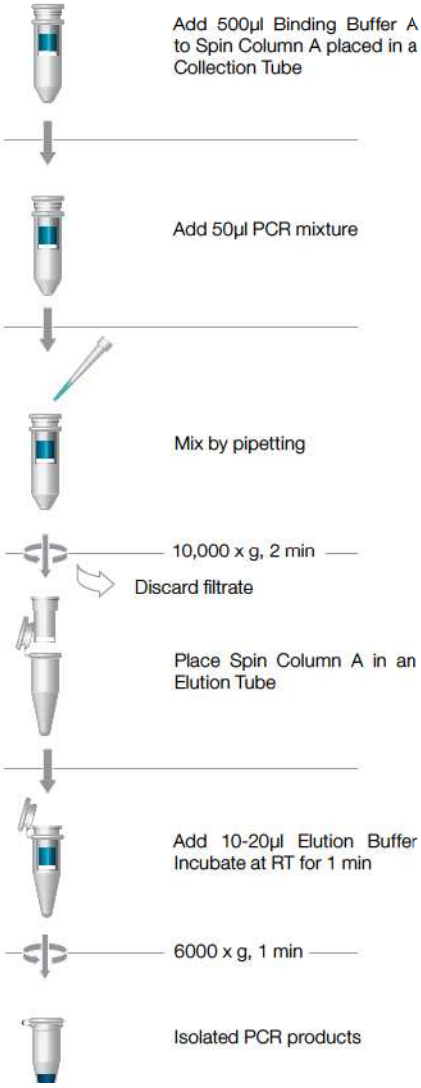
7. **Repeat step 6.**

8. **Centrifuge at maximum speed for 2 minutes to remove all traces of ethanol. Discard the Collection Tube.**

9. **Place Spin Column A into a 1.5ml Elution Tube and add 30-50µl Elution Buffer directly to the Spin Column membrane. Incubate at room temperature for 1 minute. Centrifuge at 10,000 x g (12,000rpm) for 1 minute to elute the plasmid DNA.**
   Note: Use a lower volume of Elution Buffer if a high concentration of DNA is required. Increasing the volume of water will increase the yield but decrease the concentration of DNA. Alternatively, perform two elution steps with an equal volume of Elution Buffer to increase the yield.

10. **The isolated DNA is ready for use in downstream applications or for storage at -20°C. For long term storage, freeze at -70°C.**

Isolation of PCR products



7.2 Isolation of PCR products

Before you start:

- If any of the buffers form precipitates upon storage, re-dissolve by gently warming the solution. Cool solutions down to room temperature before use.
- All steps to be carried out at room temperature, unless indicated otherwise.

1. **Place Spin Column A in a 2ml Collection Tube and add 500µl Binding Buffer A to the Spin Column.**

2. **Add up to 50µl of your PCR mixture to the Spin Column containing the Binding Buffer.**

3. **Mix well by carefully pipetting the solutions up and down a few times.**
   Note: Take care not to touch the membrane with the pipette tip. Alternatively, mix the Binding Buffer and PCR mixture in another tube and transfer to a Spin Column.

4. **Centrifuge for 2 minutes at 10,000 x g (12,000rpm).**

5. **Discard the Collection Tube and place Spin Column A in an Elution Tube.**
   Note: Avoid any contact of the Spin Column with the flow through.

6. **Add 10-20µl Elution Buffer directly on to the Spin Column membrane.**
   Note: To increase the concentration of eluted DNA, use a lower volume of Elution Buffer. The minimum recommended volume is 10µl.

7. **Incubate at room temperature for 1 minute.**
   Note: To increase the yield, extend the incubation period by up to 5 minutes.

8. **Centrifuge for 1 minute at 6000 x g (8000rpm) to elute the purified PCR products.**

9. **The isolated DNA is ready for use in downstream applications or for storage at -20°C. For long term storage, freeze at -70°C.**

Tips: If the volume of the PCR mixture is greater than 50µl, split the PCR mixture equally and add 500µl Binding Buffer to each half. Load both mixes one after another on the same Spin Column. Centrifuge the first half for 1 minute and discard the filtrate. Centrifuge the second half for 2 minutes. Follow the protocol from step 5 as described above.

8. TROUBLESHOOTING GUIDE

OBSERVATION	POSSIBLE CAUSE	RECOMMENDED SOLUTION
Low recovery	Ethanol not added to Wash Buffer	Ensure that ethanol is added to the recommended volume.
	Poor elution of DNA	Add the Elution Buffer directly to the Spin Column membrane. Prewarm Elution Buffer prior to use to increase yield.
	Ineffective solubilization of agarose gel slice	Ensure that the gel slice has dissolved completely before proceeding to the next step.
	Too much starting volume of PCR products	Follow protocol for volumes greater than 50µl on page 38.
	Insufficient mixing with Binding Buffer	Mix sample with Binding Buffer by pipetting up and down several times, or vortex sample.
DNA does not perform well in downstream applications	Ethanol carryover during elution	Increase centrifugation time for ethanol removal step.
	Salt carryover during elution	Ensure that Wash is at room temperature. Check solutions for salt precipitates. Resuspend any visible precipitate by gentle warming.
	Contamination with agarose	Wash the Spin Column once with Gel Solubilizer.
	Incorrect Elution Buffer used	If downstream reactions are sensitive to EDTA use water to elute from spin column.

A. TECHNICAL SUPPORT

For technical assistance or more information on these products, please call us on:

UK: +44 (0)20 8830 5300
US: +1 508 880 8990

DE: +49 (0)3371 68 12 29
AUST: +61 (0)2 9209 4180
INT: +44 (0)20 8830 5300

or email us at tech@bioline.com

B. ORDERING INFORMATION

PRODUCT	PACK SIZE	CAT NO.
ISOLATE Plasmid Mini Kit	10 Preps	BIO-52025
ISOLATE Plasmid Mini Kit	50 Preps	BIO-52026
ISOLATE Plasmid Mini Kit	250 Preps	BIO-52027
ISOLATE Genomic DNA Mini Kit	10 Preps	BIO-52031
ISOLATE Genomic DNA Mini Kit	50 Preps	BIO-52032
ISOLATE Genomic DNA Mini Kit	250 Preps	BIO-52033
ISOLATE Plant DNA Mini Kit	10 Preps	BIO-52034
ISOLATE Plant DNA Mini Kit	50 Preps	BIO-52035
ISOLATE Plant DNA Mini Kit	250 Preps	BIO-52036
ISOLATE PCR and Gel Kit	10 Preps	BIO-52028
ISOLATE PCR and Gel Kit	50 Preps	BIO-52029
ISOLATE PCR and Gel Kit	250 Preps	BIO-52030

C. ASSOCIATED PRODUCTS

PRODUCT	PACK SIZE	CAT NO.
ISOLATE Fecal DNA Kit	100 Reactions	BIO-52038
RiboSafe RNase Inhibitor	2500 Units	BIO-65027
Proteinase K	100mg	BIO-37037
PCR Water	10 x 10ml	BIO-37080
Agarose	500g	BIO-41025
50x TAE Buffer	5 Pouches	BIO-37103
10x TBE Buffer	10 Pouches	BIO-37104