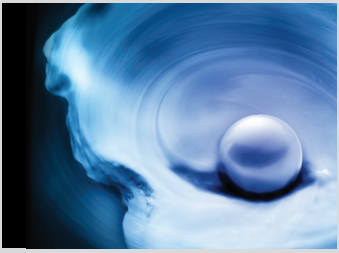


ISOLATE II
Plant DNA Kit

Product Manual

BIOLINE

A Meridian Life Science® Company

ISOLATE II Plant DNA Kit

ISOLATE II Plant DNA Kit			
1	Kit contents		04
2	Description		04
3	Storage		05
4	Safety information		05
5	Product specifications		05
6	Equipment and reagents to be supplied by user		07
7	Important notes		07
	7.1	Handling and storing starting materials	07
	7.2	Disrupting and homogenizing starting materials	07
	7.3	Lysis of plant samples	08
	7.4	Buffer preparation and parameters	10
8	Protocols		11
	8.1	Standard protocol for purifying plant genomic DNA	11
	8.2	Support protocol for purifying fungal genomic DNA	13
	8.3	Support protocol for dung, animal fecal, soil and compost samples	14
9	Troubleshooting guide		14
General Information			
A.	Hints and tips		16
B.	Technical support		16
C.	Ordering information		16
D.	Associated products		16
E.	Product warranty and disclaimer		16

1. KIT CONTENTS

COMPONENT	10 Preps	50 Preps	250 Preps
ISOLATE II Filters (violet)	10	50	250
ISOLATE II Plant DNA Spin Columns (green)	10	50	250
Collection Tubes (2 mL)	20	100	500
Lysis Buffer PA1	5 mL	25 mL	125 mL
Lysis Buffer PA2	4 mL	20 mL	100 mL
Precipitation Buffer PL3	1 mL	10 mL	25 mL
Binding Buffer PB	6 mL	30 mL	125 mL
Wash Buffer PAW1	6 mL	30 mL	125 mL
Wash Buffer PAW2† (concentrate)	6 mL	25 mL	50 mL
Elution Buffer PG	13 mL	13 mL	30 mL
RNase A (lyophilized)	1.5 mg	6 mg	2 x 15 mg
Product Manual	1	1	1
Bench Protocol Sheet	1	1	1

† Before use, add indicated volume of 96-100% ethanol and mark wash buffer bottle label.

2. DESCRIPTION

The ISOLATE II Plant DNA Kit is a simple, reliable and fast method for isolation of high-quality genomic DNA from plant cells and tissues as well as filamentous fungi. Plant samples are first disrupted/homogenized and then lysed in a highly optimized buffer system, containing chaotropic salt, denaturing agent and detergents. A choice of two lysis buffers based on the established CTAB or SDS methods are provided. Crude lysates are cleared by centrifugation and/or filtration using the ISOLATE II Filters to remove polysaccharides, insoluble plant debris and other contaminants. The cleared lysate is then mixed with the Binding Buffer and processed through an ISOLATE II Plant DNA Spin Column containing a silica membrane to which the plant genomic DNA binds. Contaminants and impurities such as salts, metabolites and cellular components are removed by simple washing steps with two different buffers. High-quality purified plant genomic DNA is then eluted in a low salt Elution Buffer or nuclease-free water. The DNA is ready-to-use for a wide variety of applications e.g. real-time PCR, next generation sequencing, restriction analysis, and array technology.

Please read this manual carefully to familiarize yourself with the ISOLATE II Plant DNA protocol before starting (also available on www.bioline.com). More experienced users can refer to the bench-top protocol for quick referencing during the procedure.

3. STORAGE

Store lyophilized RNase at 4°C on arrival (stable for up to 1 year). Reconstituted RNase I solution is stable for 12 months at -20°C. All other kit components should be stored at room temperature (18-25°C) and are stable for at least one year. Storage at lower temperatures may cause precipitation of salts.

4. SAFETY INFORMATION

When working with chemicals, always wear a suitable lab coat, gloves and safety glasses.

Buffers PA1, PA2, PB and PAW1 contain guanidine hydrochloride and/or detergents like CTAB or SDS.

For detailed information, please consult the material data safety sheets (MSDSs) available on our website at www.bioline.com.

5. PRODUCT SPECIFICATIONS

The ISOLATE II Plant DNA Kit is specially designed for the rapid and efficient isolation of extremely pure genomic DNA from plant cells and tissues or filamentous fungi. The DNA is suitable for a wide variety of applications e.g. real-time PCR and DNA sequencing. The preparation time is 30 min for 6 preps. The isolated DNA is of high-purity, yield and is free from enzymatic inhibitors (see below).

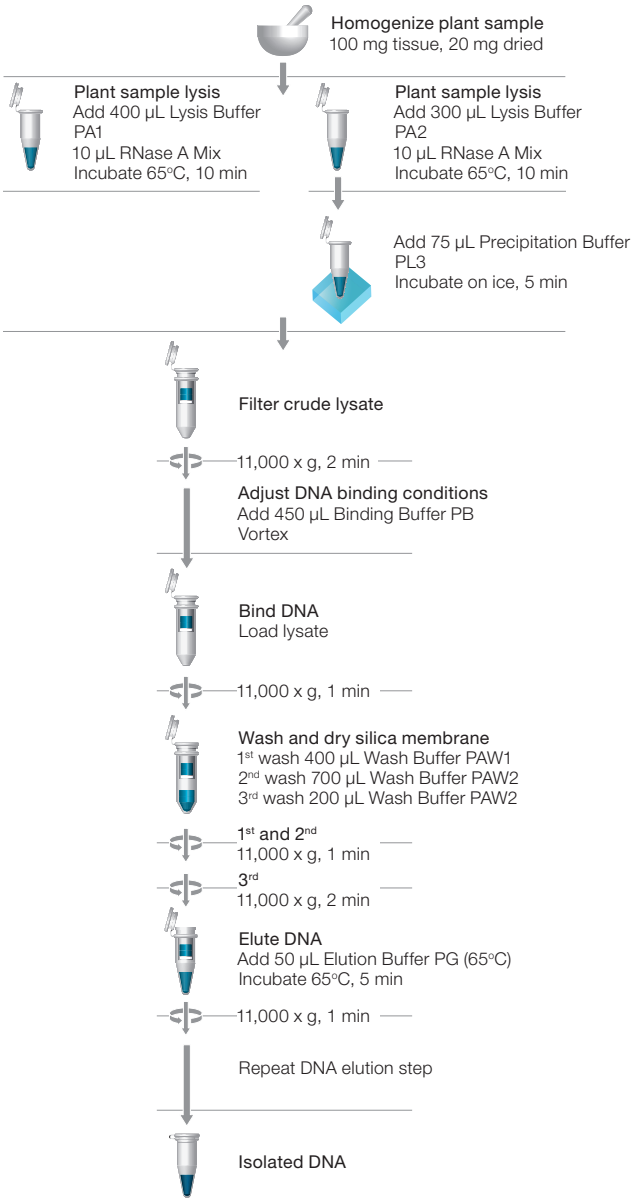
ISOLATE II PLANT DNA SPIN COLUMN SPECIFICATIONS	
Max. binding capacity	50 µg DNA
A_{260}/A_{280} ratio*	1.6–1.9
Typical yield*	1-30 µg
Elution volume	2 x 50 µL
Max. amount of starting material	
Plant tissues	100 mg (wet)/20 mg (dry)
Filamentous fungi	100 mg (wet)/20 mg (dry)

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

The following components are also included in the kit:

- ISOLATE II Filters for homogenization and to clear crude lysates.
- RNase A to eliminate RNA and to facilitate quantification of genomic DNA by photometry.
- Two highly optimized lysis buffers, PA1 and PA2 containing CTAB and SDS detergents, respectively.

Plant DNA Isolation



6. EQUIPMENT AND REAGENTS TO BE SUPPLIED BY USER

When working with chemicals, always wear a suitable lab coat, protective goggles and disposable gloves. Please consult the relevant MSDS from the product supplier for further information.

- 96–100% ethanol† (for Wash Buffer PAW2)
- Equipment for sample disruption and homogenization (section 7.2). One or more of the following are required depending on chosen method.
  - o Mortar and pestle
  - o Rotor-stator or bead-mill homogenizer
  - o Vortex mixer
  - o Liquid nitrogen
- Microcentrifuge tubes (1.5 mL)
- Disposable tips
- Pipettes
- Microcentrifuge (capable of 11,000 x g)
- Thermal heating block or water bath

† Molecular biology grade ethanol is recommended. Do not use denatured alcohol which contains unwanted additives such as methanol and acetone.

7. IMPORTANT NOTES

7.1 HANDLING AND STORING STARTING MATERIALS

Plant samples can be stored in ethanol, lyophilized/dried or frozen. Fresh material can be kept at 4°C for 24 hours but should be frozen at -20°C for longer storage.

7.2 DISRUPTING AND HOMOGENIZING STARTING MATERIALS

Due to the tough nature of plant tissue, the lysis procedure is most effective with well-homogenized, powdered samples. Suitable methods include commercial rotor-stator homogenizers or bead mills using steel or glass beads. However, we recommend grinding with a mortar and pestle in the presence of liquid nitrogen to obtain optimal yields. After homogenization and treatment of the sample with lysis buffer, the crude lysate can be cleared easily either with an ISOLATE II Filter or by centrifugation.

Disruption and homogenization using a mortar and pestle

A mortar and pestle can be used in combination with liquid nitrogen to disrupt and lyse frozen plant tissue samples. Grind the frozen tissue into a fine powder and add liquid nitrogen as necessary. It is important to ensure the sample does not thaw during or after grinding. Then transfer tissue powder with a precooled spatula into a liquid nitrogen cooled tube and allow liquid nitrogen to evaporate before closing the tube.

Disruption and homogenization using a bead mill homogenizer

Put 4-5 beads (e.g. 7 mm diameter steel beads) and plant material into a 15 mL Falcon tube. Chill the tube in liquid nitrogen and vortex for approx. 30 seconds. Repeat the cooling and vortexing procedure until all plant material is ground to a fine powder. Chill the tube once more and remove the beads by gently rolling them out or remove with a magnet. Keep the material frozen throughout the whole homogenization procedure. Nitrogen should not be added to the tube as this leads to sticking and loss of plant material attached to the beads.

Disruption and homogenization using a rotor-stator homogenizer

Rotor-stator homogenizers are only useful to disrupt soft plants in the presence of lysis buffer. To minimize foaming, keep the homogenizer submerged at all times.

7.3 LYSIS OF PLANT SAMPLES

Increasing the amount of starting material

The standard protocols of the ISOLATE II Plant DNA Kit facilitate processing of 10-1500 mg plant material. This typically yields 1-300 µg of high quality DNA. However, the amount of DNA that can be expected per mg of sample depends on both the size and genomic status (DNA ploidy). For example, 100 mg fresh wheat containing a hexaploid genome (1.7×10^{10} bp) contains 30 µg DNA, whereas the same amount of *Arabidopsis* with a smaller diploid genome (1.9×10^{8} bp) only yields 3 µg DNA.

To obtain a sufficient DNA yield, it may therefore, be advantageous to process a higher than recommended starting mass (up to 5-fold). However, to ensure complete lysis, all lysis buffer volumes (protocol step 2) must be increased proportionally and require multiple loading steps.

Selecting the optimal lysis buffer

Heterogeneity in structures of wall polysaccharides, polyphenols and other components in plants, can result in suboptimal DNA extraction or performance in downstream applications. Therefore, two different lysis buffers are provided for optimal processing, purification performance and yield with most common plant species.

The standard protocol uses Lysis Buffer PA1 based on the well-established CTAB procedure. Additionally, Lysis Buffer PA2 (SDS-based) is supplied which requires subsequent protein precipitation by potassium acetate (Precipitation Buffer PL3). For some plant species, Lysis Buffers PA1 and PA2 can be used with similar results. For most plant material, however, the differing lysis efficiency is due to the negative charge of SDS and the positive charge of CTAB.

Table 1 details some commonly used plant species and optimal lysis buffer successfully verified with the ISOLATE II Plant DNA Kit. The table should only be consulted as a rough guide. When testing a batch of material from a specific plant species for the first time, empirical testing of both buffers side-by-side is recommended in order to determine optimal lysis conditions.

Important note: *For a large number of diverse plant species, either lysis buffer generates excellent results.*

TABLE 1: PLANT SPECIES SUCCESSFULLY TESTED WITH ISOLATE II PLANT DNA KIT

Species	Organ	Lysis Buffer PA1	Lysis Buffer PA2
Plant			
Thale cress *(Arabidopsis thaliana)*	Leaves	Yes	Yes
Maize *(Zea mays)*	Leaves	Yes	Yes
Common Wheat *(Triticum aestivum)*	Leaves	Yes	Yes
Barley *(Hordeum vulgare)*	Leaves	Yes	Yes
Rice *(Oryza sativa)*	Leaves	Yes	Yes
Tomato *(Lycopersicon esculentum)*	Stems	Yes	Yes
Fungi			
Fungal (unspecified)	Mycelia	Yes	Not tested

For details of many other plant species and corresponding lysis buffers successfully tested using the ISOLATE II Plant DNA Kit, please see: www.bioline.com/isolate

7.4 BUFFER PREPARATION AND PARAMETERS

For all protocols prepare the following:

Lysis Buffer PA2

Check for precipitated SDS especially if stored below 20°C. If necessary, incubate the bottle for several minutes at 30-40°C and thoroughly mix until SDS precipitate is re-dissolved completely.

Preparing Wash Buffer PAW2 with ethanol

Add 96-100% ethanol to Wash Buffer PAW2 Concentrate: 24 mL for the 10 prep kit, 100 mL for the 50 prep kit and 200 mL for the 250 prep kit.

Important Note: *Mark bottle label to indicate ethanol was added. Wash Buffer PAW2 at is stable at room temperature (18-25°C) for at least one year*

Preparing RNase A stock solution

Reconstitute lyophilized RNase A in nuclease-free water: 150 µL for the 10 prep kit, 0.6 mL for the 50 prep kit and 1.5 mL x 2 for the 250 prep kit. Store RNase A solution at 4°C for up to 3 months. For long-term storage of up to 1 year, divide into small aliquots and store at -20°C.

Elution Parameters

Elute DNA using Elution Buffer PG (supplied). In general, two consecutive elutions increases DNA yield compared to a single elution at the same total buffer volume. This is particularly important for small buffer volumes. However, large volumes of elution buffer or the use of two consecutive elutions will result in low DNA concentration. The standard elution procedure is already optimized to yield 80–90% after two elution steps at elevated temperatures. The elution protocol can be modified to improve yield, concentration or increase elution speed:

- Standard elution: dispense 50 µL Elution Buffer PG, incubate for 5 min at 65°C and repeat (85-90% yield).
- Maximum yield: dispense 100 µL Elution Buffer PG, incubate for 5 min at 65°C and repeat (95-100% yield).
- High concentration: dispense 25 µL Elution Buffer PG, incubate for 5 min at 65°C and repeat (75% yield).
- Rapid elution: dispense 100 µL Elution Buffer PG, incubate for 1-5 min at room temperature or 65°C (60-70% yield).

8. PROTOCOLS

8.1 STANDARD PROTOCOL FOR PURIFYING PLANT GENOMIC DNA

The ISOLATE II Plant DNA Kit includes two different lysis buffers for optimal results with most common plant species. Please refer to section 7.3 for choosing the optimal lysis buffer system for your plant sample and for information on how to process more sample material than in the standard protocol.

Before you start:

- Ensure Wash Buffer PAW2 and RNase A are prepared (section 7.4)
- Preheat Elution Buffer PG to 65°C.

1 **Homogenization**

Homogenize up to 100 mg wet weight or up to 20 mg dry weight (lyophilized) plant material. Refer to homogenization methods (section 7.2).

Proceed to cell lysis using Lysis Buffer PA1 (step 2.1) or alternatively Lysis Buffer PA2 (step 2.2).

2 **Lysis**

2.1 **Cell lysis with Lysis Buffer PA1**

Transfer resulting powder to a new tube and add 400 µL Lysis Buffer PA1. Vortex mixture thoroughly.

Note: If sample does not resuspend easily e.g. due to plant powder absorbing too much buffer, add more Lysis Buffer PA1. Note that the volumes of RNase A (step 2.1) and Binding Buffer PB (step 4) have to be increased proportionally.

Add 10 µL RNase A solution and thoroughly mix sample. Incubate at 65°C for 10 min.

Note: For certain plants, increasing incubation time to 30–60 min may be required.

Proceed to step 3.

2.2 **Cell lysis with Lysis Buffer PA2**

Transfer resulting powder to a new tube and add 300 µL Lysis Buffer PA2. Vortex mixture thoroughly.

Note: If sample does not resuspend easily e.g. due to plant powder absorbing too much buffer, add more Lysis Buffer PA2. The volumes of RNase A, Precipitation Buffer PL3 (step 2.2), and Binding Buffer PB (step 4) however, have to be increased proportionally.

Add 10 µL RNase A solution and thoroughly mix sample. Incubate at 65°C for 10 min.

Note: For certain plants, increasing incubation time to 30–60 min may be required.

Add 75 µL Precipitation Buffer PL3, mix thoroughly and incubate for 5 min on ice to precipitate SDS completely.

Proceed to step 3.

3 **Filter crude lysate**

Place an ISOLATE II Filter (violet) into a new Collection Tube (2 mL) and load lysate onto column. Centrifuge for 2 min at 11,000 x g. Collect the clear flow-through and discard the ISOLATE II Filter.

Repeat the centrifugation step if not all liquid has passed through the filter.

If a pellet is visible in the flow-through, transfer the clear supernatant without disturbing the pellet to a new 1.5 mL microcentrifuge tube (not supplied).

4 **Adjust DNA binding conditions**

Add 450 µL Binding Buffer PB. Mix thoroughly by pipetting up and down 5 times or by vortexing.

5 **Bind DNA**

Place an ISOLATE II Plant DNA Spin Column (green) into a new Collection Tube (2 mL) and load sample (max. of 700 µL).

Centrifuge for 1 min at 11,000 x g and discard the flow-through.

The maximum loading capacity of the ISOLATE II Plant DNA Spin Column is 700 µL. For higher volumes repeat the loading and centrifugation steps.

6 **Wash and dry silica membrane**

- Add 400 µL Wash Buffer PAW1 to the ISOLATE II Plant DNA Spin Column. Centrifuge for 1 min at 11,000 x g and discard flow-through.

Note: Although washing with Wash Buffer PAW1 increases purity, it can in certain cases reduce the final yield slightly.

- Add 700 µL Wash Buffer PAW2 to the ISOLATE II Plant DNA Spin Column. Centrifuge for 1 min at 11,000 x g and discard flow-through.
- Add another 200 µL Wash Buffer PAW2 to the ISOLATE II Plant DNA Spin Column. Centrifuge for 2 min at 11,000 x g in order to remove wash buffer and to dry the silica membrane completely.

7 **Elute DNA**

Place the ISOLATE II Plant DNA Spin Column into a new 1.5 mL microcentrifuge tube (not supplied).

Pipette 50 µL Elution Buffer PG (65°C) onto the membrane. Incubate the ISOLATE II Plant DNA Spin Column for 5 min at 65°C. Centrifuge for 1 min at 11,000 x g to elute the DNA.

Repeat this step with another 50 µL Elution Buffer PG (65°C) and elute into the same tube.

Note: To achieve maximum yield or higher concentrations refer to section 7.4 for alternative elution procedures.

8.2 SUPPORT PROTOCOL FOR PURIFYING FUNGAL GENOMIC DNA

Additional reagents/components to be supplied by the user:

- Ethanol (96–100%)
- Chloroform
- Micropestle
- Siliconized glass beads or sea sand

1 **Homogenize sample**

Wash 50–200 mg mycelium (fresh weight) or 50-200 mg material from a fruiting body of macro fungi in ethanol. Mycelium can be obtained from a liquid culture or scraped off (with or without agar) from the surface of a solid medium.

Submerge sample completely in ethanol and mix carefully. In most cases, short washes in ethanol are sufficient; however, overnight incubation can sometimes increase DNA yield (long-term storage in ethanol is also possible). Remove ethanol by pipetting and squeezing the sample.

2 **Cell lysis**

Transfer sample into a 1.5 mL microcentrifuge tube (not supplied). Add 150 mg siliconized glass beads or sea sand and 200 µL Lysis Buffer PA1. Homogenize sample using a micropestle and vortex regularly. Add additional 100 µL Lysis Buffer PA1 and continue to homogenize the sample.

Note: If the sample cannot be easily handled because e.g. the sample material soaks up too much buffer, additional Lysis Buffer PA1 can be added. The volume of Binding Buffer PB (step 4) however, has to be increased proportionally.

Optional: If the sample is rich in RNA or protein, we recommend adding 10 µL RNase A and/or Proteinase K (5–10 mg/mL stock solution, see ordering information), respectively, to the PA1 lysis solution in order to minimize contaminants.

Incubate for 10 min at 65°C.

Note: For some fungi it might be advantageous to increase the incubation time to 30–60 min.

Add 100 µL chloroform. Vortex for 10s and separate phases by centrifugation for 15 min at 20,000 x g. Pipette the top aqueous layer into a new 1.5 mL microcentrifuge tube (not supplied).

3 **Filter lysate**

Place ISOLATE II Filter in Collection Tube (2 mL), apply lysate and centrifuge for 1 min at 11,000 x g. This step reduces solution turbidity and viscosity.

If a visible pellet forms, transfer supernatant whilst avoiding the pellet to a new 1.5 mL microcentrifuge tube (not supplied).

Alternatively, pass lysate ≥5 times through a 20 gauge (0.9 mm) needle and syringe.

Proceed with step 3 from section 8.1.

8.3 SUPPORT PROTOCOL FOR DUNG, ANIMAL FECAL, SOIL AND COMPOST SAMPLES

Additional reagents/components to be supplied by user:

- Mortar and pestle or bead mill homogenizer
- Sea sand (siliconized)
- Extraction buffer: 2 M NaCl, 20 mM EDTA, 100 mM Tris/HCl, 2% (w/v) CTAB, 2% (w/v) polyvinylpyrrolidone (MW 40,000), pH 8.0

1 **Homogenize sample**

In a petri dish, weigh 5 g soil or 2 g fecal sample and add extraction buffer until sample is completely soaked. Heat in a microwave oven (400W) for several seconds until extraction buffer foams.

Extraction buffer may be added to maintain the sample in a slushy state.

2 **Cell lysis**

Transfer sample into a bead mill or mortar. Add 0.5 mL sand and disrupt the sample (see step 2 from section 8.2 for sample lysis/homogenization details).

3 **Filtration / Clarification of lysate**

Transfer the homogenized sample into a centrifuge tube and centrifuge for 10 min at 5,000 x g. Pipette 300 µL of the clear supernatant into a new 1.5 mL microcentrifuge tube (not supplied).

Proceed with step 3 (filter crude lysate) from section 8.1.

9. TROUBLESHOOTING GUIDE

CLOGGED COLUMNS	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Too much sample material	Do not use more sample material than recommended in the protocol. Centrifuge large amounts of sample material before loading it onto the ISOLATE II Filter column. Make sure the cleared lysate is absolutely free of suspended matter before loading it onto the ISOLATE II Plant DNA Spin Column. Increase centrifugation speed and time. Increase volume of Lysis Buffer PA1 or PA2.
Incomplete lysis	Increase volume of Lysis Buffer PA1 or PA2. Increase incubation time to overnight.
DEGRADED DNA	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Sample contaminated with DNase	If another elution buffer than Elution Buffer PG is used, ensure it is free of DNase activity, for example addition of 1 mM EDTA or heating the buffer to 70°C for 10 min.

Centrifugation speed was too high	Centrifuge at a maximum speed of 11,000 x g. Higher velocities may cause DNA shearing.
LOW DNA YIELD	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Homogenization of plant material insufficient	For most species we recommend grinding with steel beads or mortar and pestle (see section 7.2). For disruption of the cell wall it is important to homogenize the plant material thoroughly until the sample is ground to a fine powder. Instead of freezing in liquid nitrogen, the sample can also be lyophilized and ground at room temperature.
Suboptimal lysis buffer used	Lysis efficiencies of Lysis Buffer PA1 (CTAB) and Lysis Buffer PA2 (SDS) differ and depend on individual plant species (see Table 1). Test both buffers side-by-side to determine optimal detergent system for your plant sample.
Suboptimal lysis buffer volume was used	Cell lysis might be insufficient and too much DNA might get lost during lysate clarification i.e. dry material absorbs too much lysis buffer. Add additional lysis buffer and increase the volume of Binding Buffer PB proportionally.
Extraction of DNA from plant material during lysis was insufficient	Increase incubation time in lysis buffer (up to overnight).
Reagents not applied correctly	Ensure steps for reagent preparation are followed (see section 7.4).
Suboptimal elution	The DNA can either be eluted in higher volumes or by repeating the elution step up to three times (see section 7.4). Incubate ISOLATE II Plant DNA Spin Column with Elution Buffer PG at 65°C for at least 5 minutes. Check the pH of the elution buffer, which should be in the range of pH 8.0– 8.5. To ensure correct pH, use supplied Elution Buffer PG (5 mM Tris/HCl, pH 8.5).
SUBOPTIMAL PERFORMANCE OF EXTRACTED GENOMIC DNA IN ENZYMATIC REACTIONS	
POSSIBLE CAUSE	RECOMMENDED SOLUTION
Carry-over of ethanol or salt	Ensure the final two wash steps are performed with Wash Buffer PAW2 and that the membrane was dried according to the protocol.
Elution of DNA with buffers other than Elution Buffer PG e.g. TE Buffer	We recommend elution with supplied Elution Buffer PG (5 mM Tris/HCl, pH 8.5), as chemicals such as EDTA found in other buffers can interfere with downstream applications.

A. TECHNICAL SUPPORT

For technical assistance or more information on these products, please email us at tech@bioline.com

B. ORDERING INFORMATION

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II Plant DNA Kit	10 Preps	BIO-52068
ISOLATE II Plant DNA Kit	50 Preps	BIO-52069
ISOLATE II Plant DNA Kit	250 Preps	BIO-52070
Proteinase K	100 mg	BIO-37037

C. ASSOCIATED PRODUCTS

PRODUCT	PACK SIZE	CAT NO.
ISOLATE II Genomic DNA Kit	50 Preps	BIO-52065
ISOLATE II RNA Plant Kit	50 Preps	BIO-52077
MyTaq™ HS DNA Polymerase	250 Units	BIO-21111
SensiFAST™ SYBR No-ROX Kit	200 Reactions	BIO-98002

D. PRODUCT WARRANTY AND DISCLAIMER

Bioline warrants that its products will conform to the standards stated in its product specification sheets in effect at the time of shipment. Bioline will replace free of charge any product that does not conform to the specifications. This warranty limits Bioline's liability only to the replacement of the product.

NOTES

PM0816V2.0

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