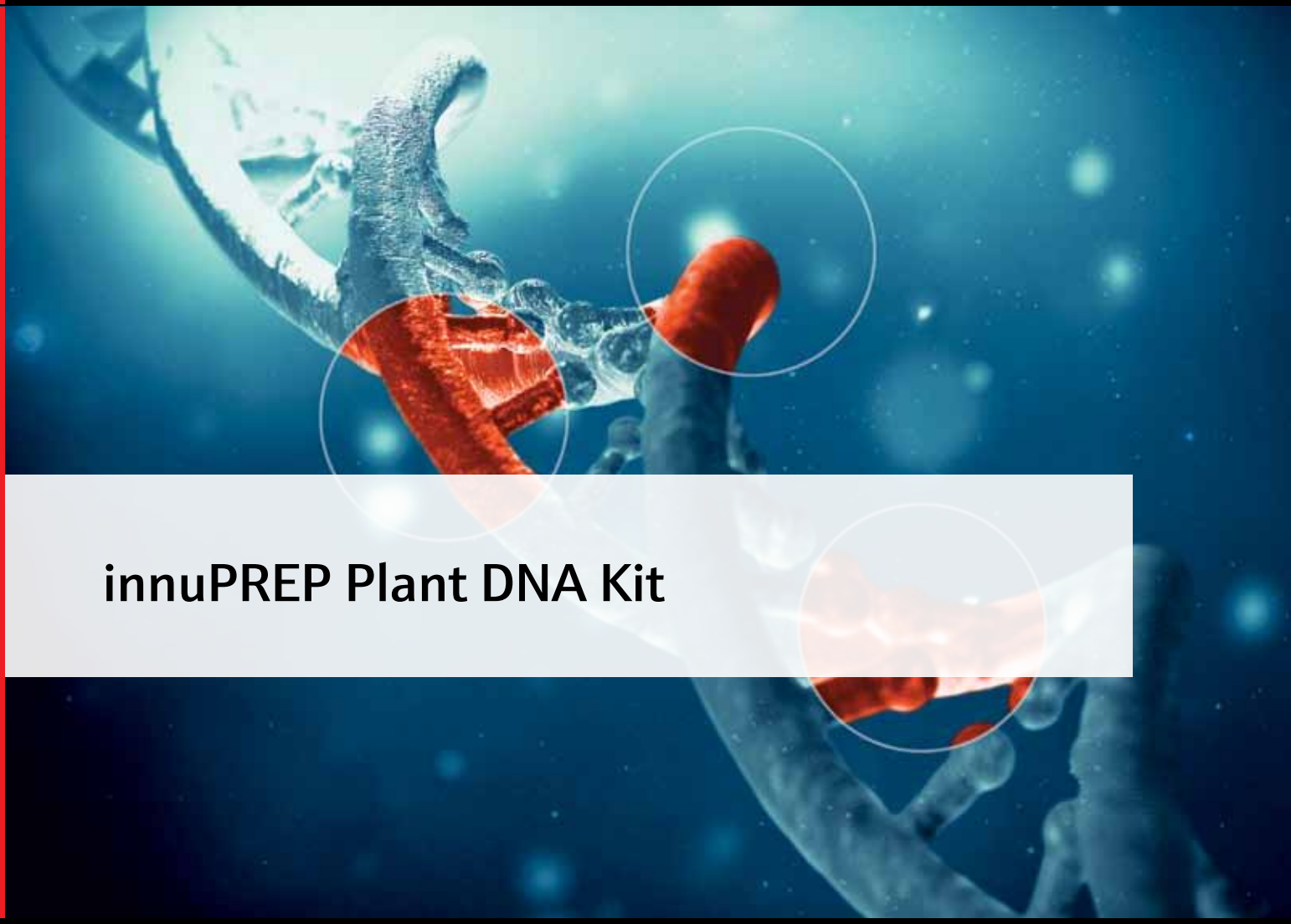
Instructions for Use
Life Science Kits & Assays



innuPREP Plant DNA Kit

analytikjena
An Endress+Hauser Company

Order No.:
845-KS-1060010 10 reactions
845-KS-1060050 50 reactions
845-KS-1060250 250 reactions

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1. Introduction

1.1 Intended use

The innuPREP Plant DNA Kit has been designed for simple, reliable and fast isolation of genomic DNA (gDNA) from different kinds of plant material. The extraction procedure is based on a new kind of patented technology (called DC chemistry). The innuPREP Plant DNA Kit is optimized for the rapid preparation of highly pure gDNA from plant material.

The protocol is based on using mortar and pestle and liquid nitrogen for homogenization. The homogenization is followed by a lysis step. For lysis of plant material the kit contains three different Lysis Solutions. These are Lysis Solution SLS, Lysis Solution OPT and Lysis Solution CBV. All three Lysis Solutions contain a mixture of chaotropic and anti-chaotropic salts (DC-Technology), detergents and other additives. Following the homogenization the lysates will be cleared by a centrifugation based precipitation and/or filtration using a Prefilter to remove polysaccharides, contaminations and residual cellular debris. The clear flow-through is mixed with Binding Solution SBS to create conditions for optimal binding of gDNA to the silica membrane of the Spin Filter. After washing steps the gDNA can be eluted from the membrane by using Elution Buffer (provided) or RNase-free water (not provided) and is ready-to-use for subsequent downstream applications.

The innuPREP Plant DNA Kit allows processing of up to 50–100 mg (dry weight) or 120–180 mg (wet weight) starting material.

The kit has been tested for isolation of gDNA from leafs, fruits, woods, needles as well as seeds. The starting material can be fresh or frozen. Depending on the individual sample typical yields are in the range from 3-25 µg DNA (more yield are also possible).

CONSULT INSTRUCTION FOR USE
This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert.

1.2 Notes on the use of this manual

For easy reference and orientation, the manual uses the following warning and information symbols as well as the shown methodology:

Symbol	Information
REF	**REF** Catalogue number.
Σ N	**Content** Contains sufficient reagents for <N> reactions.
15°C 30°C	**Storage conditions** Store at room temperature or shown conditions respectively.
	Consult instructions for use This information must be observed to avoid improper use of the kit and the kit components.
	Expiry date
LOT	**Lot number** The number of the kit charge.
	Manufactured by Contact information of manufacturer.
	For single use only Do not use components for a second time.
	Note / Attention Observe the notes marked in this way to ensure correct function of the kit and to avoid operating errors for obtaining correct results.

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → "Notes on the use of this manual" p. 3).
- Working steps are numbered.

2. Safety precautions

NOTE

Read through this chapter carefully prior to guarantee your own safety and a trouble-free operation.

Follow all the safety instructions explained in the manual, as well as all messages and information, which are shown.

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.

FOR SINGLE USE ONLY!

This kit is made for single use only!

ATTENTION!

Don't eat or drink components of the kit!

The kit shall only be handled by educated personal in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries. Analytik Jena AG has not tested the liquid waste generated during using the kit for potential residual infectious components. This case is highly unlikely, but cannot be excluded completely. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.

Please observe the federal, state and local safety and environmental regulations. Follow the usual precautions for applications using extracted nucleic acids. All materials and reagents used for DNA or RNA isolation should be free of DNases or RNases.

ATTENTION!
Do not add bleach or acidic components to the waste after sample preparation!

NOTE
Emergency medical information in English and German can be obtained 24 hours a day from:

Poison Information Center, Freiburg / Germany
Phone: +49 (0)761 19 240.

For more information, please ask for the material safety data sheet (MSDS).

3. Storage conditions

Store lyophilized Proteinase K at 4 °C to 8 °C. All other components of the innuPREP Plant DNA Kit should be stored dry at room temperature (15 °C to 30 °C). When stored at room temperature, the kit is stable until the expiration date printed on the label on the kit box. If there are any precipitates within the provided solutions solve these precipitates by careful warming. Before every use make sure that all components have room temperature. For further information see chapter “Kit components” (→ p. 8).

4. Functional testing and technical assistance

The Analytik Jena AG guarantees the correct function of the kit for applications as described in the manual. Each lot of innuPREP Plant DNA Kit is tested against predetermined specifications to ensure consistent product quality in accordance with the ISO-certified Quality Management System of Analytik Jena AG.

We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the innuPREP Plant DNA Kit or other Analytik Jena AG products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 36 41 / 77 94 00. For other countries please contact your local distributor.

5. Product use and warranty

The kit is not designed for the usage of other starting materials or other amounts of starting materials than those, referred to in the manual (→ “Intended use” p. 2) (→ “Product specifications” p. 10). Since the performance characteristics of our kits have not been validated for any specific application. Analytik Jena AG kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA’ 88 regulations in the U.S. or equivalents in other countries.

All products sold by the Analytik Jena AG are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately

NOTE

For research use only!

6. Kit components

STORGAGE CONDITIONS

All components except **Proteinase K** are stored at room temperature.

	Σ 10	Σ 50	Σ 250
REF	845-KS-1060010	845-KS-1060050	845-KS-1060250
Lysis Solution SLS	5 ml	25 ml	120 ml
Lysis Solution OPT	5 ml	25 ml	120 ml
Lysis Solution CBV	5 ml	25 ml	120 ml
Precipitation Buffer P	2 ml	4 x 2 ml	30 ml
Binding Solution SBS	5 ml	15 ml	60 ml
Proteinase K	For 1 x 0.3 ml working solution	For 1 x 1.5 ml working solution	For 4 x 1.5 ml working solution
Washing Solution MS (conc.)	6 ml	24 ml	2 x 60 ml
Elution Buffer	2 x 2 ml	15 ml	60 ml
Prefilter	10	50	5 x 50
Spin Filter	10	50	5 x 50
Receiver Tubes	50	5 x 50	25 x 50
Elution Tubes	10	50	5 x 50
Manual	1	1	1

	Σ 10	Σ 50	Σ 250
Initial steps	**Washing Solution MS (conc.)** Add 14 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed!	**Washing Solution MS (conc.)** Add 56 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed!	**Washing Solution MS (conc.)** Add 140 ml of 96-99.8 % ethanol to the bottle and mix thoroughly. Keep the bottle always firmly closed!
	Proteinase K Dissolve Proteinase K by addition of 0.3 ml of ddH_2O, mix thoroughly and store as described below!	**Proteinase K** Dissolve Proteinase K by addition of 1.5 ml of ddH_2O, mix thoroughly and store as described below!	**Proteinase K** Dissolve Proteinase K by addition of 1.5 ml of ddH_2O, mix thoroughly and store as described below!

IMPORTANT

Store lyophilized as well as dissolved **Proteinase K** at 4 ˚C to 8 ˚C.

Components not included in the kit

- ddH_2O for dissolving **Proteinase K**
- RNase A (100 mg/ml); optional
- 1.5 ml tubes
- 2.0 ml tubes; optional
- 96–99.8 % ethanol

NOTE

Use only absolute/pure ethanol, NO methylated or denatured alcohol!

7. Product specifications

1. Starting material:

   Fresh, frozen or dried plant material (up to 100 mg dry weight or 180 mg wet weight)

2. Time for isolation:

   Approximately 30–40 minutes

3. Binding capacity:

   > 50 µg DNA

4. Typical yield:

   - 3–25 µg
   - Depending on the kind and initial amount of the starting material

8. GHS classification

Component	Hazard contents	GHS Symbol	Hazard phrases	Precaution phrases
Lysis Solution SLS	Ammonium chloride 10–<25 % Cetrimonium bromide 1–<2.5 %	 *Warning*	319, 400	101, 102, 103, 280, 273, 264, 305+351+338, 501
Precipitation Buffer P	Acetic acid 10–≤25 %	 *Warning*	319	101, 102, 103, 280, 305+351+338, 362, 332+313, 302+352
Binding Solution SBS	Propan-2-ol 50–100 % Polyethylene glycol octylphenol ether 25–50 %	 *Danger*	225, 315, 318, 336, 411	101, 102, 103, 210, 303+361+353, 305+351+338, 310, 405, 501
Proteinase K	Proteinase, engyodontium album	 *Danger*	315, 319, 334, 317, 335	101, 102, 103, 261, 280, 305+351+338, 342+311, 405, 501

CAUTION

DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

8.1 Hazard phrases

225	Highly flammable liquid and vapor.
315	Causes skin irritation.
317	May cause an allergic skin reaction.
318	Causes serious eye damage.
319	Causes serious eye irritation.
334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
335	May cause respiratory irritation.
336	May cause drowsiness or dizziness.
400	Very toxic to aquatic life.
411	Toxic to aquatic life with long lasting effects.

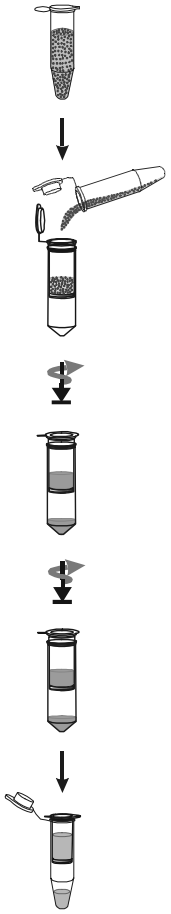
8.2 Precaution phrases

101	If medical advice is needed, have product container or label at hand.
102	Keep out of reach of children.
103	Read label before use.
210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
261	Avoid breathing dust/fume/gas/mist/vapors/spray.
264	Wash thoroughly after handling.
273	Avoid release to the environment.
280	Wear protective gloves/protective clothing/ eye protection/face protection.
310	Immediately call a POISON CENTER/doctor.
362	Take off contaminated clothing.
405	Store locked up.
501	Dispose of contents/container in accordance with local/regional/national/international regulations.
302+352	IF ON SKIN: Wash with plenty of water.
332+313	If skin irritation occurs: Get medical advice/attention.
342+311	If experiencing respiratory symptoms: Call a POISON CENTER/doctor.
303+361+353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
305+351+ 338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

9. Recommended steps before starting

- Heat thermal mixer or water bath at 65 °C.
- Ensure that the **Washing Solution MS** and **Proteinase K** have been prepared according to the instruction (→ "Kit components" p. 8).
- Centrifugation steps should be carried out at room temperature.
- Avoid freezing and thawing of starting material.

10. General procedure for DNA extraction



Lysis of starting material and optional pre-filtration or precipitation with subsequent pre-filtration by using a Prefilter

Binding of DNA onto Spin Filter

Washing of the bound DNA

Elution of DNA

11. Homogenization and lysis of plant samples

If plant tissue will not be used immediately after harvesting, it can be stored in liquid nitrogen, lyophilized/dried or frozen. Fresh material can be kept at 4 °C to 8 °C for 24 hours but should be frozen at -22 °C to -18 °C or for longer storage at -80 °C for later processing. Ground tissue powder can also be stored at -80 °C. Alternatively, tissue can be dried or lyophilized after harvesting to allow storage at room temperature (15 °C to 30 °C). To ensure DNA quality, samples should be completely dried within 24 hours of collection.

We recommended to collect young materials (e.g., leaves, needles) since they contain more cells per weight and therefore result in higher yields. In addition, young leaves and needles contain smaller amounts of polysaccharides and polyphenolics and are therefore easier to handle.

Complete and quick disruption of starting material is essential to ensure high DNA yields and to avoid DNA degradation. The lysis procedure is most effective with well-homogenized, powdered samples. Suitable methods include any type of commercial homogenizers (rotor-stator homogenizer) or bead mills (e.g. SpeedMill PLUS, Analytik Jena AG) using ceramic beads. However, we recommend grinding with a mortar and pestle in the presence of liquid nitrogen to obtain optimal yields. When using tissues other than leaves, the disruption method may require optimization to ensure maximum DNA yield and quality. After homogenization and treatment of the sample with lysis solution, the crude lysate can be cleared easily either with Prefilters or by centrifugation.

Disruption of starting material using a mortar and pestle

Use mortar and pestle to grind the plant material in the presence of liquid nitrogen. Freeze plant material in liquid nitrogen and be careful during homogenization, because do not let the sample thaw at any time. We recommend precooling the used laboratory equipment. Grind frozen plant sample to a fine powder and refill mortar with liquid nitrogen to keep the sample frozen, if necessary. Use precooled tubes for sample storage until

lysis step, but make sure no liquid nitrogen is transferred or all nitrogen has evaporated before closing the tube.

Disruption of starting material using bead mill homogenizers

Use 0.5 g ceramic beads (e.g. 2.4–2.6 mm ceramic beads–Lysis Tube P, Analytik Jena AG) for plant material and leaves or 4–5 steel beads in a mixture (e.g. 4.7 mm diameter steel beads–Lysis Tube Z, Analytik Jena AG) for seeds, rice and needles. Pipette 50 µl ddH_2O to the plant material and vortex for about 30 seconds (e.g. SpeedMill PLUS, Analytik Jena AG). Repeat the chilling and vortexing procedure until the entire plant material is ground to a fine solution.

It is also possible to chill the tube in liquid nitrogen. After the homogenization, describe above, chill the tube once more and remove the beads by rolling them out gently or using a magnet. Keep the material frozen throughout the whole homogenization procedure. Do not add nitrogen to the tube since this leads to sticking and loss of plant material attached to the beads.

Disruption of starting material using a Rotor-stator homogenizer

Rotor-stator homogenizers are only useful to disrupt soft plants in the presence of lysis solution. Keep homogenizer submerged at all times to reduce foaming.

Lysis of plant samples

Increasing the amount of starting material

The standard protocols of innuPREP Plant DNA Kit allow processing of 50–100 mg (dry weight) or 120–180 mg (wet weight) of plant material. This usually yields 1-25 µg of high quality DNA. However, the amount of DNA that can be expected per mg of sample depends on the size and ploidy of the genome.

To obtain sufficient DNA yield, it might be advantageous to process a higher than the recommended sample mass. However, to ensure a complete lysis, all lysis solution volumes of protocol step 2 have to be increased proportionally and require multiple loading steps.

Selecting the optimal lysis solution system

Plants are very heterogeneous and contain varying amounts of polyphenols, acidic components, or polysaccharides which can lead to suboptimal DNA extraction or performance in downstream applications. Therefore, three different lysis solutions are provided for optimal processing, purification performance, high yields and an excellent DNA quality for the most common plant species.

The standard protocol uses **Lysis Solution SLS**, containing CTAB as detergent component. Additionally, the SDS based **Lysis Solution OPT** is provided which requires subsequent precipitation step to remove all impurities by **Precipitation Buffer P**. For some plant species **Lysis Solutions SLS** and **OPT** can be used with similar results. In these cases please make a choice for the easiest protocol. Further the **Lysis Solution CBV** has been optimized for isolation of gDNA from seeds, but can also be used for other plant materials.

In order to find optimal lysis conditions when using a certain plant sample for the first time, it is recommended to do side-by-side preparations of one batch of homogeneously ground material with the three different lysis solutions and make a decision for the best one (regarding yield, quality or other relevant parameters). The following example of application illustrates the effects of different lysis solutions on yield and quality of the extracted gDNA.

Application example:

Isolation of gDNA from rice (*Oryza sativa*) using the three different Lysis solutions SLS, OPT and CBV. The spectrophotometric measurement shows different results depending on the lysis solution used. It compares with the Ct-values of the detection of a plant specific genome by Real-Time PCR.

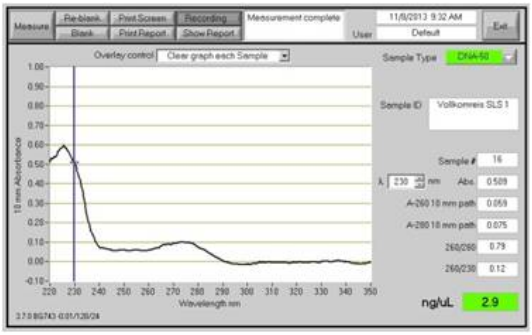


Fig. 1: Concentration of DNA with **Lysis Solution SLS**: 2.9 ng/µl and purity (A_{260}/A_{230}): 0.79.

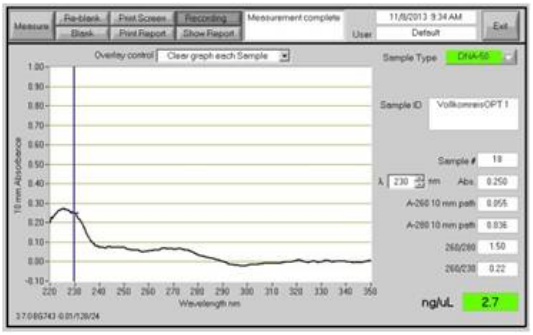


Fig. 2: Concentration of DNA with **Lysis Solution OPT**: 2.7 ng/µl and purity (A_{260}/A_{230}): 1.50.

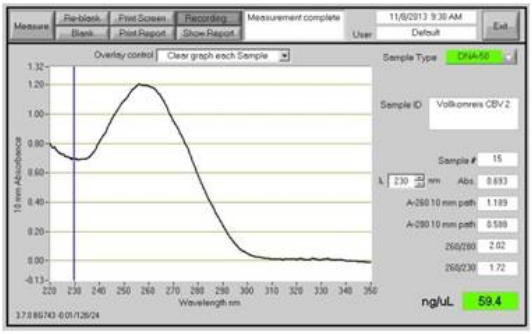


Fig. 3: Concentration of DNA with **Lysis Solution CBV**: 59.4 ng/µl and purity (A_{260}/A_{230}): 2.02.

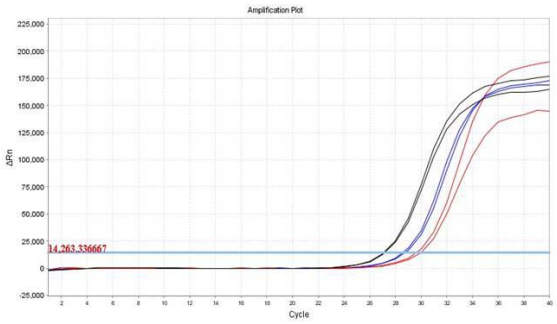


Fig. 4: Ct-values of Real-Time PCR with **Lysis Solution SLS** (red): 29.8, OPT (blue): 28.8 and CBV (black): 27.0.

IMPORTANT NOTE

For a large variety of plant species, either lysis solution generates good results.

12. Protocols: DNA isolation from plant material

IMPORTANT NOTE

The innuPREP Plant DNA Kit include three different lysis solutions for optimal results with most common plant species. Please refer to section "Homogenization and lysis of plant samples" (p. 15) for choosing the optimal lysis solution system for your individual plant sample.

In order to prevent confusions when you perform several preparations in parallel, label the tubes and the filters!

1. Homogenization of about **50–100 mg** of **starting material** by a pestle under liquid N_2.

   Commercially available equipment for homogenization (e.g. SpeedMill PLUS) also can be used. Alternatively grinding of plant material with sand is also possible (→ "Homogenization and lysis of plant samples", p. 15).

   See also: innuSPEED Plant DNA Kit

NOTE

Use 120–180 mg of starting material if extraction from material which is very wet or contains more water.

Proceed with cell lysis using **Lysis Solution SLS** (Protocol 1), **Lysis Solution OPT** (Protocol 2) or **Lysis Solution CBV** (Protocol 3).

12.1 Protocol 1: gDNA from plant using Lysis Solution SLS

1. Transfer the plant powder or other homogenized starting material in a 1.5 ml or 2.0 ml reaction tube. Add **400 µl Lysis Solution SLS and 20 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 seconds.

   Incubate at 65 °C for approx 30 minutes (longer incubation is also possible, up to 60 minutes).

NOTE
We recommend to use a shaking platform (thermomixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally 3–4 times during lysis step. No shaking will reduce the lysis efficiency.

2. Transfer the sample onto a Prefilter located in a Receiver Tube and centrifuge the tube at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Prefilter.

   Don't discard the Receiver Tube with the filtrate!

NOTE
To remove RNA from the sample (if necessary) add 4 µl of RNase A solution (100 mg/ml) to the filtrate, vortex shortly and incubate for 5 minutes at room temperature.

3. Add **200 µl Binding Solution SBS** to the lysed sample (filtrate from step 3), mix by pipetting up and down several times.

NOTE
It is important that the sample and the **Binding Solution SBS** are mixed vigorously to get a homogeneous solution.

4. Apply the sample to a Spin Filter located in a new Receiver Tube. Close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 2 minutes.

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time. For higher sample volumes repeat the loading step.

Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

5. Open the Spin Filter and add **650 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
6. Open the Spin Filter and add **650 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
7. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the Receiver Tube.
8. Place the Spin Filter into an Elution Tube. Carefully open the cap of the Spin Filter and add **100–200 µl Elution Buffer**. Incubate at room temperature for 1 minute. Centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. A second elution step (e.g. 100 µl + 100 µl) will increase the yield of extracted DNA.

NOTE
The DNA can be eluted with a lower or a higher volume of **Elution Buffer** (depends on the expected yield of genomic DNA). Elution with lower volumes of **Elution Buffer** increases the final concentration of DNA. Store the extracted DNA at 4 °C to 8 °C. For long time storage placing at -22 °C to -18 °C is recommended.

12.2 Protocol 2: gDNA from plant using Lysis Solution OPT

1. Transfer the plant powder or other homogenized starting material in a 1.5 ml or 2.0 ml reaction tube. Add **400 µl Lysis Solution OPT**, mix vigorously by pulsed vortexing for 5 seconds.

   Incubate at 65 °C for approx 30 minutes (longer incubation is also possible, up to 60 minutes).

NOTE
We recommend to use a shaking platform (thermomixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally 3–4 times during lysis step. No shaking will reduce the lysis efficiency.

2. Add **100 µl Precipitation Buffer P** and vortex the sample for 5 seconds. Incubate at room temperature for 5 minutes and centrifuge at maximum speed for 5 minutes.
3. Transfer the clear supernatant onto a Prefilter located in a Receiver Tube and centrifuge the tube at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Prefilter.

   Don't discard the Receiver Tube with the filtrate!

NOTE
If there is a pellet after centrifugation, don't discard the pellet. Transfer the supernatant carefully into a new reaction tube.

NOTE
To remove RNA from the sample (if necessary) add 4 µl of RNase A solution (100 mg/ml) to the filtrate, vortex shortly and incubate for 5 minutes at room temperature.

4. Add **200 µl Binding Solution SBS** to the lysed sample (filtrate from step 3), mix by pipetting up and down several times.

NOTE
It is important that the sample and the **Binding Solution SBS** are mixed vigorously to get a homogeneous solution.

5. Apply the sample to a Spin Filter located in a new Receiver Tube. Close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 2 minutes.

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time. For higher sample volumes repeat the loading step.

Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

6. Open the Spin Filter and add **650 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
7. Open the Spin Filter and add **650 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
8. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the Receiver Tube.

9. Place the Spin Filter into an Elution Tube. Carefully open the cap of the Spin Filter and add **100–200 µl Elution Buffer**. Incubate at room temperature for 1 minute. Centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. A second elution step (e.g. 100 µl + 100 µl) will increase the yield of extracted DNA.

NOTE
The DNA can be eluted with a lower or a higher volume of **Elution Buffer** (depends on the expected yield of genomic DNA). Elution with lower volumes of **Elution Buffer** increases the final concentration of DNA. Store the extracted DNA at 4 °C to 8 °C. For long time storage placing at -22 °C to -18 °C is recommended.

12.3 Protocol 3: gDNA from plant using Lysis Solution CBV

1. Transfer the plant powder or other homogenized starting material in a 1.5 ml or 2.0 ml reaction tube. Add **400 µl Lysis Solution CBV and 20 µl Proteinase K**, mix vigorously by pulsed vortexing for 5 seconds.

   Incubate at 65 °C for approx 30 minutes (longer incubation is also possible, up to 60 minutes).

NOTE
We recommend to use a shaking platform (thermomixer, water bath or another rocking platform) for a continuous shaking of the sample. Vortex the sample optionally 3–4 times during lysis step. No shaking will reduce the lysis efficiency.

2. Add **100 µl Precipitation Buffer P** and vortex the sample for 5 seconds. Incubate at room temperature for 5 minutes and centrifuge at maximum speed for 5 minutes.
3. Transfer the clear supernatant onto a Prefilter located in a Receiver Tube and centrifuge the tube at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Prefilter.

   Don't discard the Receiver Tube with the filtrate!

NOTE
If there is a pellet after centrifugation, don't discard the pellet. Transfer the supernatant carefully into a new reaction tube.

NOTE
To remove RNA from the sample (if necessary) add 4 µl of RNase A solution (100 mg/ml) to the filtrate, vortex shortly and incubate for 5 minutes at room temperature.

4. Add **200 µl Binding Solution SBS** to the lysed sample (filtrate from step 3), mix by pipetting up and down several times.

NOTE
It is important that the sample and the **Binding Solution SBS** are mixed vigorously to get a homogeneous solution.

5. Apply the sample to a Spin Filter located in a new Receiver Tube. Close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 2 minutes.

NOTE
If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time. For higher sample volumes repeat the loading step.

Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

6. Open the Spin Filter and add **650 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.
7. Open the Spin Filter and add **650 µl Washing Solution MS**, close the cap and centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. Dis-

card the Receiver Tube with the filtrate. Place the Spin Filter into a new Receiver Tube.

8. Centrifuge at max. speed for 2 minutes to remove all traces of ethanol. Discard the Receiver Tube.
9. Place the Spin Filter into an Elution Tube. Carefully open the cap of the Spin Filter and add **100–200 µl Elution Buffer**. Incubate at room temperature for 1 minute. Centrifuge at 11,000 x g (~11,000 rpm) for 1 minute. A second elution step (e.g. 100 µl + 100 µl) will increase the yield of extracted DNA.

NOTE
The DNA can be eluted with a lower or a higher volume of **Elution Buffer** (depends on the expected yield of genomic DNA). Elution with lower volumes of **Elution Buffer** increases the final concentration of DNA. Store the extracted DNA at 4 °C to 8 °C. For long time storage placing at -22 °C to -18 °C is recommended.

13. Troubleshooting

Problem / probable cause	Comments and suggestions
Clogged Spin Filter	
Insufficient lysis and/or too much starting material	Increase lysis time. Increase centrifugation speed. After lysis centrifuge the lysate to pellet unlysed material. Reduce amount of starting material.
Low amount of extracted DNA	
Insufficient lysis	Select the optimal lysis solutions by side by side preparation of one batch of homogeneously starting material. Increase lysis time. Reduce amount of starting material. Overloading of Spin Filter reduces yield!
Incomplete elution	Prolong the incubation time with **Elution Buffer** to 5 minutes or repeat elution step once again. Take a higher volume of **Elution Buffer** or elute in two steps.
Insufficient mixing with **Binding Solution SBS**	Mix sample with **Binding Solution SBS** by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.
Low concentration of extracted DNA	
Too much **Elution Buffer**	Elute the DNA with lower volume of **Elution Buffer**.
Degraded or sheared DNA	
Incorrect storage of starting material	Ensure that the starting material is frozen immediately in liquid N_2 or in minimum at-20 °C and is stored continuously at -80 °C! Avoid thawing of the material.
Old material	Old material often contains degraded DNA.
RNA contaminations of extracted DNA	RNase A digestion

14. Related Products

Name	Amount	Order No.
Nucleid acid purification		
innuPREP Proteinase K	6 mg	845-CH-0010006
	30 mg	845-CH-0010030
Products for PCR & Gel Electrophoresis		
innuTaq DNA Polymerase (5 U/µl)	500 U	845-EZ-1000500
50x inNucleotide Mix (12,5 mM)	2x 0.5 ml	845-AS-9000100
inNucleotide Set (100 mM)	4x 0.25 ml	845-AS-1100250
innuMIX rapidPCR MasterMix	100 rxn	845-AS-1600100
	200 rxn	845-AS-1600200
innuMIX Standard PCR MasterMix	100 rxn	845-AS-1700100
	200 rxn	845-AS-1700200
innuMIX Green PCR MasterMix	100 rxn	845-AS-1400100
	200 rxn	845-AS-1400200
innuSTAR 100 bp DNA Ladder Express	500 µl	845-ST-1010100
	5x 500 µl	845-ST-1010500
innuSTAR 1 kb DNA Ladder Express	500 µl	845-ST-1020100
	5x 500 µl	845-ST-1020500
6x Loading Dye Bromophenol Blue	3x 1.0 ml	845-ST-3010003
	6x 1.0 ml	845-ST-3010006
6x Loading Dye Orange G	3x 1.0 ml	845-ST-4010003
	6x 1.0 ml	845-ST-4010006
Products for qPCR		
innuMIX qPCR MasterMix Probe	100 rxn	845-AS-1200100
	200 rxn	845-AS-1200200
innuMIX qPCR MasterMix SyGreen	100 rxn	845-AS-1300100
	200 rxn	845-AS-1300200

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Subject to changes in design and scope of delivery as well as further technical development!