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SPEEDTOOLS PLANT DNA EXTRACTION KIT

Designed for the Rapid Isolation of Highly Pure DNA from Plants and Food Material of Plant Origin

Instructions for Use

(Cat. No. 21.171/2)

PLEASE READ THE INSTRUCTIONS FOR USE THOROUGHLY BEFORE USING THE KIT, ESPECIALLY IF YOU ARE NOT FAMILIAR WITH THE PROTOCOL.

1. BASIC PRINCIPLE

SPEEDTOOLS PLANT DNA EXTRACTION KIT is a system designed for the rapid isolation of high quality DNA from plant (leaves, roots, fruits or seeds) and food material of plant origin (fresh, frozen or dried material).

The technology presented for SPEEDTOOLS PLANT DNA EXTRACTION KIT combines the advantages of a high efficient lysis buffer with a powerful novel technique for isolation of nucleic acids characterised by the use of non-chaotropic salts for the binding of nucleic acids to a solid phase[1].

In a first step the starting material is homogenized to a fine powder which is treated with the lysis Buffer L (based on the established CTAB procedure), and Proteinase solution. In order to remove contaminants and residual cellular debris lysis mixture is cleared by filtration onto the provided columns. The clear flow-through is mixed with the binding Solution P to create the appropriate conditions for binding of DNA in the column membrane. After loading this mixture onto the column, the contaminants are washed away using subsequent washing steps with Buffer W1 and Buffer W2. The DNA is finally eluted from the column with the elution Buffer E.

2. KIT CONTENTS

SPEEDTOOLS PLANT DNA EXTRACTION KIT		
	Cat. No. 21.171 50 Preps	Cat. No. 21.172 250 Preps
BUFFER L Lysis buffer	30 ml	5 x 30 ml
SOLUTION P Binding solution	15 ml	5 x 30 ml
BUFFER W1 Wash buffer I	30 ml	5 x 30 ml
BUFFER W2 Wash buffer II	18 ml	5 x 18 ml
PROTEINASE K (lyophilized)	1 vial	5 vials
BUFFER E Elution buffer	15 ml	15 x 2 ml
BIOTOOLS PREFILTER	50	5 x 50
BIOTOOLS BINDING COLUMN	50	5 x 50
1.5 ml COLLECTING TUBES	50	5 x 50
2 ml COLLECTING TUBES	100	5 x 100
PROTOCOL	1	5 x 1

3. KIT SPECIFICATIONS

The isolation protocol as well as all reagents are optimized to provide high yield and purity of the isolated genomic DNA.

The Kit uses a new patented technology for binding nucleic acids onto a column without hazardous chaotropic or high salt buffers. Moreover, the number of steps and the hands-on time necessary for the whole procedure are reduced to a minimum. Up to 100 mg of fresh plant material (wet weight) can be processed in about 20 min after lysis

The kit allows the isolation of highly pure genomic DNA with an A260/280-ratio between 1.60 and 2. Typical DNA yield for the Kit depend on the individual sample (amount and kind of starting material), values up to 50 μg are achieved.

[1] The used technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

The obtained DNA is ready-to-use in subsequent reactions like PCR, RFLP analysis, restriction enzyme digestion, sequencing, cloning or southern blot.

General Characteristics of the Kit

Sample Size	Up to 100 mg
Yield	Up to 50 µg
Ratio A_{260}:A_{280}	1.6 - 2
Elution Volume	100 µl
Time	20 min (after lysis time)
Spin Column Type	mini

4. PREPARATION OF WORKING SOLUTIONS AND STORAGE CONDITIONS

NOTE

Buffer L, Buffer W1 and Proteinase K include harmful components. Solution P is highly flammable and irritant. Wear disposable gloves, a suitable coat and protective goggles when working with the Kit.

All **Kit components except Proteinase K can be stored at room temperature (20-25°C)** and are stable up to twelve months after fabrication under these conditions. The **lyophilized Proteinase K must be stored at 2-8°C** once dissolved stored Proteinase K solution at – 20°C.

Before starting any protocol with **SPEEDTOOLS PLANT DNA EXTRACTION KIT** prepare:

- **PROTEINASE K:** Add 1 ml of sterile bidistilled water to dissolve lyophilized Proteinase K. **Store Proteinase K solution at –20°C**. Repeating freezing and thawing will reduce the activity of Proteinase K dramatically, therefore dividing the Proteinase K solution into aliquots and store them at –20°C is recommended.
- **BUFFER W1 (Wash Buffer I):** Add 30 ml of ethanol (96-100%) to the concentrate buffer W1 provided with the Kit. Mix thoroughly and keep the bottle always firmly closed. Store at room temperature.
- **BUFFER W2 (Wash Buffer II):** Add 42 ml of ethanol (96-100%) to the concentrate buffer W2 provided with the Kit. Mix thoroughly and keep the bottle always firmly closed. Store at room temperature.

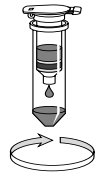
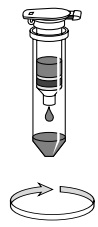
For its use equilibrate Kit components at room temperature. If there are any precipitates within the provided solutions solve these precipitates by careful warming.

5. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Appropriate equipment for sample homogenization if required e.g. mortar and pestle, commercial homogenizers, steel beads, microwave, etc.
- Water bath / Incubator / Heating block
- Ethanol 96-100%
- Vortex
- Microcentrifuge and tubes
- Sterile bidistilled water
- Opcional: RNase A (10 mg/ml)

6. STANDARD PROTOCOL FOR DNA EXTRACTION FROM PLANT OR FROM FOOD FROM PLANT ORIGIN

Before starting with the protocol set tree incubators or heating blocks at 65ºC. Equilibrate the needed amount of Buffer E to 65°C. Prepare Proteinase K solution, Buffer W1 and Buffer W2 (see section 4).

STEP	DESCRIPTION		
1	**HOMOGENIZATION OF THE STARTING MATERIAL** ***As plant cell wall is very robust, the lysis procedure is most effective with well-homogenized, powdered samples. Suitable methods include grinding with a mortar and pestle in the presence of liquid nitrogen or any type of commercial homogenisers.*** Homogenize about 60 mg of starting material. If the starting material contains high water content e.g. fruits and algaes use a larger sample 120-180 mg.		**HOMOGENIZE SAMPLE (60 mg)**
2	**SAMPLE LYSIS AND PRECLEANING** Transfer the powder into a 1.5 ml reaction tube. Add **400 µl Lysis Buffer L** and **20 µl Proteinase K.** Vortex shortly and incubate for **30 min at 65ºC** under continuously shaking. For each preparation take a **Prefilter** and place the filter into a 2 ml Collecting Tube. Transfer the lysate onto the filter and centrifuge for 1 min at 12,000 rpm. Remove the Prefilter and keep the filtrate. ***To remove RNA from the sample add to the filtrate 40 µl of RNase A (10 mg/ml), vortex briefly and incubate for 5 min at room temperature***	 	**+** **400 µl BUFFER L** **20µl PROTEINASEK** **Vortex** 65°C, 30 min with shaking Transfer into a **Prefilter** *1 min, 12,000 rpm*
3	**ADJUST DNA BINDING CONDITIONS** Add **200 µl Solution P** to each tube containing the filtrate and **vortex** thoroughly.		**+** **200 µl** **SOLUTION P** **vortex**
4	**BIND DNA** Place a **Binging column** into a 2 ml Collecting Tube. **Transfer the filtrate suspension onto the column** and **incubate for 1 min.** Centrifuge **1 min at 12,000 rpm. Discard the filtrate** and place the column back into the Collecting Tube.		**Load filtrate solution into a Binding column** *1 min, 12,000 rpm*
5	**WASHING** **1st Wash** Add **550 µl Buffer W1**. Centrifuge **1 min at 12,000 rpm**. **Discard flow-through** and place the column back into the Collecting Tube. **2nd Wash and 3rd Wash** Add **550 µl Buffer W2**. Centrifuge **1 min at 12,000 rpm**. **Discard** the **filtrate** and place the column into the Collecting Tube. Repeat the washing step once again. To remove residual ethanol from the filter of the binding column centrifuge **2 min at 12,000 rpm**.		**+** **550 µl BUFFER W1** *1 min, 12,000 rpm* **+** **550 µl BUFFER W2** *1 min, 12,000 rpm* **+** **550 µl BUFFER W2** *1 min, 12,000 rpm* *2 min, 12,000 rpm*
6	**ELUTION OF HIGHLY PURE DNA** Place the column in a new 1.5 ml collecting tube. Add **100 µl prewarmed elution Buffer E (65°C)**. Dispense Buffer E directly onto the membrane. Incubate at room temperature for 3 min. Centrifuge **1 min at 10,000 rpm**. The eluate contains your pure DNA sample. ***NOTE: The minimum elution volume of Buffer E is 50 µl. If large amount of DNA is expected the elution volume can be increased 100-200 µl.***		**100 µl BUFFER E (65ºC)** **Incubate 3 min RT** *1 min, 10,000 rpm*

7. TROUBLESHOOTING

Problem	Possible cause and suggestions
Binding column clogged	***Insufficient lysis* and/or too much starting material** • Increase lysis time • Increase centrifugation speed or time • Reduce amount of starting material
Low content of extracted DNA	***Insufficient lysis*** • Increase lysis time • Reduce amount of starting material. Overloading of spin filter reduces yield • Don't forget the addition of Proteinase K ***Incomplete elution*** • Prolong the incubation time with Elution Buffer E to 5-10 min • Repeat elution step once again • Take higher volume of Elution Buffer E ***Insufficient mixing with binding solution*** • Mix sample with Binding Solution P completely by pipetting or by vortexing prior to transfer the sample onto the binding column
Low concentration of extracted DNA	***Too much elution Buffer E*** • Elute the DNA with lower volume of Elution Buffer E
Degraded or shared DNA	***Incorrect storage of starting material or old material*** • Ensure that the starting material is fresh or stored under appropriate conditions. Avoid repeating thawing and freezing of the material • Old material often contains degraded DNA. Avoid repeated freeze/thaw cycles of starting material
Contamination of extracted DNA with RNA	• RNase A digestion
DNA does not perform well in downstream-applications	***Ethanol carryover during elution*** • Increase time for removing ethanol ***Salt carryover during elution*** • Ensure that wash buffers are at room temperature. Check up wash buffers for salt precipitates. If there are many precipitates, solve these precipitates by warming carefully

8. SAFETY INSTRUCTIONS

The following components of the SPEEDTOOLS PLANT DNA EXTRACTION KIT include hazardous contents. Wear gloves and goggles and follow the safety instructions given in this section.

Reagent	Hazard Contents	Hazard Symbol		Risk Phrases	Safety Phrases
Solution P (binding solution)	Isopropanol	 F Highly flammable  Xi Irritant	Highly flammable. Irritating to eyes. Vapors may cause drowsiness and dizziness Keep out of the reach of the children. Keep container tightly closed. Keep away from sources of ignition-No smoking. Avoid contact with skin and eyes, rinse immediately with plenty of water and seek medical advice.	R11-R36-R67	S2-S7-S16-S24/26
Buffer L (lysis buffer)	Ammonium chloride, CTAB	 Xn Harmful  N Dangerous for the environment	Harmful if swallowed. Irritating to eyes, respiratory system and skin. Risk of serious damage to the eyes. May cause sensitization by inhalation, by skin contact. Risk of explosion if heated under confinement. May cause cancer, heritable genetic damage, birth defects. Danger of serious damage to health by prolonged exposure. May cause cancer by inhalation. Very toxic to aquatic organisms. Do not breathe dust. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear eye/face protection.	R22-R36/38-R41/50	S22-S26-S39
Buffer W1 (Wash buffer 1)	Guanidine isothiocyanate	 Xn Harmful	Harmful by inhalation, in contact with skin and if swallowed. Contact with acids liberates very toxic gas. Keep out of the reach of the children. Keep away from food, drink and animal feedingstuffs	R20/22-R32	S2-S13
Proteinase K	Proteinase K, lyophilised	 Xn Harmful	Irritating to eyes,respiratory system and skin. May cause sensitisation by inhalation. Keep out of the reach of the children. Do not breathe dust. Avoid contact with the skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing and gloves.	R 36/38-42	S2-S22-S24-S26-S36/37

9. ORDERING INFORMATION

SPEEDTOOLS KIT	50 PREPS	250 PREPS
SPEEDTOOLS **DNA** EXTRACTION KIT	Cat. No. 21.131	Cat. No. 21.132
SPEEDTOOLS **TISSUE DNA** EXTRACTION KIT	Cat. No. 21.136	Cat. No. 21.137
SPEEDTOOLS **RNA VIRUS** EXTRACTION KIT	Cat. No. 21.141	Cat. No. 21.142
SPEEDTOOLS **FOOD DNA** EXTRACTION KIT	Cat. No. 21.176	Cat. No. 21.177
SPEEDTOOLS **PLANT DNA** EXTRACTION KIT	Cat. No. 21.171	Cat. No. 21.172
SPEEDTOOLS **TOTAL RNA** EXTRACTION KIT	Cat. No. 21.211	Cat. No. 21.212
SPEEDTOOLS **PCR CLEAN-UP** KIT	Cat. No. 21.201	Cat. No. 21.202
SPEEDTOOLS **PLASMID DNA** PURIFICATION KIT	Cat. No. 21.221	Cat. No. 21.222

10. PRODUCT USE RESTRICTION AND WARRANTY

Following is a list of warning and precautions. For further information, please refer to the Material Safety Data Sheet (MSDS), available in our webpage (www.biotools.eu), or by request to our Technical Dpt. (info@biotools.eu).

1. Product for research purposes only.
2. The kit includes documentation stating specifications and other technical information. Follow the instructions in order to obtain highly pure plant DNA. The user is responsible to validate the performance of the Kit for any particular use, since the performance characteristics of the kit have not been validated for any specific application. The Kit 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.
3. BIOTOOLS warrants to meet the stated specifications of the kit. Any product not fulfilling the specifications included in the product sheet will be replaced.
4. BIOTOOLS obligation and purchaser's rights under this warranty are limited to the replacement by BIOTOOLS of any product that is shown defective in fabrication, and that must be returned to BIOTOOLS, freight prepaid, or at BIOTOOLS' option, replacement of the purchasing price.
5. BIOTOOLS does not warrant against damages or defects arising in shipping and handling (transport insurance for customers excluded). Any complaint on damaged goods during transport must be directed to the handling or transport agent.
6. BIOTOOLS has no responsibility for damages, whether direct or indirect, incidental or consequential of improper or abnormal use of this product. Nor has any responsibility for defects in products or components not manufactured by BIOTOOLS, or against damages resulting from such non-BIOTOOLS components or products.
7. BIOTOOLS makes no other warranty of any kind whatsoever, and specifically disclaims and excludes all other warranties of any kind or nature whatsoever, directly or indirectly, express or implied, including, without limitation, as to the suitability, reproductively, durability, fitness for a particular purpose or use, merchantability, condition, or any other matter with respect to BIOTOOLS products.
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9. Product claims are subject to change. Therefore please contact our Technical Service Team for the most up-to-date information. You may also contact your local distributor for general scientific information.
10. Applications mentioned in BIOTOOLS literature are provided for informational purposes only. BIOTOOLS does not warrant that all applications have been tested in BIOTOOLS laboratories using BIOTOOLS products. BIOTOOLS does not warrant the correctness of any of those applications. For more information contact our Technical Dept (info@biotools.eu).

Manufactured by:

BIOTOOLS, Biotechnological & Medical Laboratories, S.A. has been evaluated and certified to accomplish ISO 9001:2000 requirements for the following activities: Research and development of biotechnology products and manufacture of biotechnology and in vitro products.
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