

# BIOTOOLS

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## **SPEEDTOOLS PLANT DNA EXTRACTION KIT**

*Designed for the Rapid Isolation of Highly Pure DNA  
from Plants*

### **Instructions for Use** **(Ref. 21.170M/1/2)**

**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 tissue using two optimized lysis buffer systems based on the established CTAB and SDS methods.

The plant samples are homogenized by mechanical treatment. Then the DNA can be extracted with Buffer L1 or L2 containing chaotropic salts, denaturing agents and detergents. Crude lysates should be cleared by centrifugation and/or filtration using the filters provided with the kit in order to remove polysaccharides, contaminations and residual cellular debris. The clear flow-through is mixed with Buffer B to create conditions for optimal binding of DNA to the silica membrane. After loading this mixture onto the spin column, contaminants are washed away using Buffer W1 and W2. The genomic DNA can finally be eluted with Buffer E or nuclease-free water and is ready-to-use for subsequent reactions

## 2. KIT CONTENTS

SPEEDTOOLS PLANT DNA EXTRACTION KIT			
	Ref. 21.170M 10 Preps	Ref. 21.171 50 Preps	Ref. 21.172 5 x 50 Preps
Buffer L1 Lysis Buffer	5 ml	25 ml	5 x 25 ml
Buffer L2 Lysis Buffer	4 ml	20 ml	5 x 20 ml
Buffer P Precipitation Buffer	1 ml	10 ml	5 x 10 ml
Buffer B Binding Buffer	5 ml	30 ml	5 x 30 ml
Buffer W1 Wash Buffer	5 ml	30 ml	5 x 30 ml
Buffer W2 Wash Buffer	4 ml	25 ml	5 x 25 ml
Buffer E Elution Buffer	2 ml	13 ml	5 x 13 ml
RNase A (Lyophilized)	1,1 mg	6 mg	5 x 6 mg
Filters (violet rings)	10	50	5 x 50
Binding Columns (green rings)	10	50	5 x 50
2 ml Collection Tubes	20	100	5 x 100
USER MANUAL	1	1	5 x 1

## 3. KIT SPECIFICATIONS

The isolation protocol and all reagents have been optimised to provide high yield and purity of the isolated genomic DNA. Up to 100mg of fresh plant material or up to 20mg of dry material can be processed in about 30 minutes. This usually yields 1-30µg of high quality DNA with an A260/280-ratio between 1.8 and 1.9.

RNase A is included to remove RNA and to allow photometric quantification of pure genomic DNA.

The optimized Buffer B and the chaotropic Buffer W1 completely remove proteins, RNA, metabolites and other PCR inhibitors so the eluted DNA is ready-to-use for subsequent reactions like PCR, restriction analysis, Southern Blot, etc...

General Characteristics of the Kit	
Sample Size	Up to 100 mg of plant material or up to 20 mg of dry plant material
Yield	1 - 30 µg (depends on the amount and kind of starting material)
Ratio A <sub>260</sub> :A <sub>280</sub>	1.8 – 1.9
Elution Volume	2 x 50 µl
Time	30 min (after lysis)
Spin Column Type	mini

#### 4. SAMPLING AND STORAGE OF STARTING MATERIAL

Plant samples can be stored in ethanol, lyophilized or frozen. Fresh material can be kept at 4°C for one day but should be frozen at -20°C for longer storage. Multiple thawing and freezing before isolating the DNA should be avoided.

#### 5. YIELD OF GENOMIC DNA

The amount of purified DNA in the kit procedure from plant material depends on sample source, transport conditions, storage and age of the sample.

#### 6. PREPARATION OF WORKING SOLUTIONS AND STORAGE CONDITIONS

**NOTE:** *Buffer L1, L2, Buffer B and Buffer W1 contain guanidine hydrochloride and/or detergents like CTAB or SDS so wear gloves and goggles when working with the kit.*

**CAUTION:** *Buffer B and Buffer W1 contain guanidine hydrochloride which can form highly reactive compounds when combined with bleach (sodium hypochlorite). DO NOT add bleach or acidic solutions directly to the sample-preparation waste.*

All **Kit components** should be stored at **room temperature** (18-25°C); they are stable for at least 12 months (from the manufacturing date) under these conditions.

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

##### I. Buffer L1 and Buffer L2:

- ✓ Check for precipitated detergent especially after storage at temperatures below 20°C. If necessary incubate the bottle for several minutes at 30 – 40°C and mix well until the precipitate is re-dissolved completely

##### II. BUFFER W2:

- ✓ **10 Preps Format:** Add 16 ml of molecular grade ethanol (96-100%) (Not provided) and mix thoroughly. The Buffer is stable at room temperature (18° - 25°C) for at least one year
- ✓ **50 Preps Format:** Add 100 ml of molecular grade ethanol (96-100%) (Not provided) and mix thoroughly. The Buffer is stable at room temperature (18° - 25°C) for at least one year

- ✓ **5 x 50 Preps Format:** Add 100 ml of molecular grade ethanol (96-100%) (Not provided) to each bottle of buffer W2 and mix thoroughly. The Buffer is stable at room temperature (18° - 25°C) for at least one year

### III. **RNase A:**

- ✓ **10 Preps Format:** Add 110µl H<sub>2</sub>O and mix thoroughly. Store the RNase A solution at **4°C** for up to 3 months. For longer storage (up to 1 year), the RNase solution should be divided into small aliquots and store at **-20°C**
- ✓ **50 Preps Format:** Add 600µl H<sub>2</sub>O and mix thoroughly. Store the RNase A solution at **4°C** for up to 3 months. For longer storage (up to 1 year), the RNase solution should be divided into small aliquots and store at **-20°C**
- ✓ **5 x 50 Preps Format:** Add 600µl H<sub>2</sub>O to each bottle and mix thoroughly. Store the RNase A solution at **4°C** for up to 3 months. For longer storage (up to 1 year), the RNase solution should be divided into small aliquots and store at **-20°C**


## 7. 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
- Molecular Grade Ethanol 96-100%
- Vortex
- Microcentrifuge and tubes
- Sterile bidistilled water

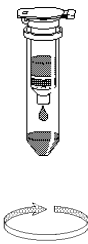

## 8. STANDARD PROTOCOL: DNA extraction from fresh or dried plant material

Before starting with the protocol set an incubator or a heating block at 65°C. Heat the needed amount of Buffer E to 65°C. Prepare Buffer W2 and RNase A according to section 6.

*NOTE: The standard protocol uses Buffer L1, which is based on the established CTAB procedure. Additionally, the SDS based Lysis Buffer (Buffer L2) is provided which requires subsequent protein precipitation by potassium acetate (Buffer P). For a large variety of plant species both lysis buffers allow good results but 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 both lysis buffers.*

STEP	DESCRIPTION		
1	<p><b>HOMOGENIZE SAMPLE</b></p> <p><i>As plant cell wall is very robust, the lysis procedure is most effective with well-homogenised, powdered samples. Suitable methods include grinding with a mortar or any type of commercial homogenisers.</i></p> <p><b>Homogenize up to 100mg wet weight or up to 20 mg dry weight (lyophilized) plant material.</b></p> <p><b>Proceed with cell lysis using Buffer L1 (step 2a) or alternatively Buffer L2 (step 2b)</b></p>		<p><b>HOMOGENIZE SAMPLES (100mg)</b></p>

2	CELL LYSIS		
2a	<p>Transfer the resulting powder to a new tube and add 400µl of <b>Buffer L1</b>. Vortex the mixture thoroughly. If the sample can not be resuspended easily additional Buffer L1 can be added. Note that the volumes of RNase A (step 2a) and Buffer B (step 4) have to be increased proportionally</p> <p>Add 10µl of RNase A solution and mix the sample thoroughly</p> <p>Incubate the suspension for 10 min at 65°C (For some plants it might be advantageous to increase the incubation time to 30 – 60min)</p> <p><b>PROCEED WITH STEP 3</b></p>		<p>400 µl BUFFER L1 Vortex</p> <p>+</p> <p>10µl RNase A</p> <p>65°C, 10 min.</p>
2b	<p><b>ALTERNATIVELY</b></p> <p>Transfer the resulting powder to a new tube and add 300µl of <b>Buffer L2</b>. Vortex the mixture thoroughly. If the sample can not be resuspended easily additional Buffer L2 can be added. Note that the volumes of RNase A, Buffer P (step 2b) and Buffer B (step 4) have to be increased proportionally.</p> <p>Add 10µl of <b>RNase A</b> solution and mix the sample thoroughly</p> <p>Incubate the suspension for 10 min at 65°C (For some plants it might be advantageous to increase the incubation time to 30 – 60min)</p> <p>Add <b>75µl of Buffer P</b>, mix thoroughly and incubate for 5 minutes on ice to precipitate SDS completely.</p> <p><b>PROCEED WITH STEP 3</b></p>		<p>300µl BUFFER L2 Vortex</p> <p>+</p> <p>10µl RNase A</p> <p>65°C, 10 min.</p> <p>+</p> <p>75µl Buffer P</p> <p>5 min. on ice</p>
3	<p><b>FILTRATION AND ADJUST DNA BINDING CONDITIONS</b></p> <p>Place a <b>Filter</b> (violet ring) into a new <b>collection tube</b> (2ml) and load the lysate onto the column. Centrifuge for 2 min at 11,000g. Collect the clear-flow-through and discard the filter</p> <p>If not all liquid has passed the filter, repeat the centrifugation step.</p> <p>Add <b>450µl Buffer B</b> and mix thoroughly by pipetting up and down or by vortexing</p>		<p>LOAD THE LYSATE ONTO THE FILTER</p> <p>2 min 11,000g</p> <p>+</p> <p>450µl BUFFER B</p>
4	<p><b>BIND DNA</b></p> <p>Place a Binding Column (green ring) into a new Collection Tube (2ml) and load a maximum of 700µl of the sample</p> <p>Centrifuge for 1 minute at 11,000g and discard the Flow-Through</p> <p>The maximum loading capacity of the Binding column is 700µl. For higher sample volumes repeat the loading step</p>		<p>LOAD 700µl OF THE LYSATE ONTO THE COLUMN (Binding Column)</p> <p>1 min, 11,000g</p>

5	<p><b>WASH AND DRY SILICA MEMBRANE</b></p> <p><b>1<sup>st</sup> Wash</b> Add 400µl of Buffer W1 to the Binding Column (green ring). Centrifuge for 1 minute at 11,000g and discard flow-through. Place the binding column again into the collection Tube</p> <p><b>2<sup>nd</sup> Wash</b> Add 700µl of Buffer W2 to the Binding Column (green ring). Centrifuge for 1 minute at 11,000g and discard flow-through. Place the binding column again into the collection Tube</p> <p><b>3<sup>rd</sup> Wash</b> Add another 200µl of Buffer W2 to the Binding Column. Centrifuge for 2 minutes at 11,000g in order to remove wash buffer and dry the silica membrane completely.</p>		<p>+ 400 µl BUFFER W1 1 min, 11,000g</p> <p>+ 700 µl BUFFER W2 1 min, 11,000g</p> <p>+ 200 µl BUFFER W2 2 min, 11,000g</p>
6	<p><b>ELUTE DNA</b></p> <p>Place the Binding Column into a new 1,5ml microcentrifuge tube (not provided)</p> <p>Pipette 50µl of Buffer E (65°C) onto the membrane. Incubate the Binding Column for 5 minutes at 65°C. Centrifuge for 1 minute at 11,000g to elute the DNA</p> <p>Repeat this step with another 50µl of Buffer E (65°C) and elute into the same tube.</p> <p><b>NOTE: Buffer E doesn't contain EDTA. If DNA degradation is observed after storage of purified DNA, adjust EDTA in Buffer E to 1mM before elution.</b></p>		<p>50 µl BUFFER E (65°C)</p> <p>Incube 5 min a 65°</p> <p>1 min, 11,000g</p> <p>Repita el Proceso</p>

## 9. TROUBLESHOOTING

Problem	Possible cause and suggestions
Binding column clogged	<p><b>Insufficient lysis and/or too much starting material</b></p> <ul style="list-style-type: none"> <li>• Increase lysis time</li> <li>• Increase centrifugation speed or time</li> <li>• Reduce amount of starting material</li> <li>• Use more Buffer L1 or L2</li> </ul>
Low amount of extracted DNA	<p><b>Insufficient lysis</b></p> <ul style="list-style-type: none"> <li>• Increase lysis time (up to overnight)</li> <li>• Use more Buffer L1 or L2</li> <li>• Reduce amount of starting material (overloading of spin filter reduces yield)</li> <li>• Lysis efficiencies of Buffer L1 (CTAB) and Buffer L2 (SDS) are different and depend on the plant species. Try both buffers in a side-by-side purification to find the best detergent system to lyse your plant material.</li> </ul> <p><b>Homogenization of plant material wasn't sufficient</b></p> <ul style="list-style-type: none"> <li>• As plant cell wall is very robust, the lysis procedure is most effective with well-homogenised, powdered samples. Suitable methods include grinding with a mortar or any type of commercial homogenisers</li> </ul> <p><b>Incomplete elution</b></p> <ul style="list-style-type: none"> <li>• Prolong the incubation time with Buffer E to 5-10 min or repeat the elution step (the elution step can be repeated up to three times)</li> <li>• Take higher volume of Buffer E</li> </ul> <p><b>Suboptimal Buffer B volume was used</b></p> <ul style="list-style-type: none"> <li>• Increase Buffer B volume proportionally if more lysis buffer (Buffer L1 or L2) was used.</li> </ul> <p><b>Too much elution Buffer E</b></p> <ul style="list-style-type: none"> <li>• Elute the DNA with lower volume of Elution Buffer E</li> </ul>
Degraded or shared DNA	<p><b>Incorrect storage of starting material or old material</b></p> <ul style="list-style-type: none"> <li>• Ensure that the starting material is fresh (old material often contains degraded DNA) or stored under appropriate conditions. Avoid repeating thawing and freezing of starting material</li> </ul> <p><b>Sample is contaminated with DNase</b></p> <ul style="list-style-type: none"> <li>• Adjust Buffer E to 1mM EDTA</li> </ul> <p><b>Centrifugation Speed was too high</b></p> <ul style="list-style-type: none"> <li>• Centrifuge at a maximum speed of 11,000g. Higher velocities may lead to shearing of the DNA</li> </ul>
DNA does not perform well in downstream-applications	<p><b>Ethanol carryover during elution</b></p> <ul style="list-style-type: none"> <li>• Make sure the last two wash steps were done with Buffer W2 and the membrane was dried according to the protocol</li> </ul>

## 10. ORDERING INFORMATION

SPEEDTOOLS KIT	10 PREPS	50 PREPS	250 (5 x 50) PREPS
SPEEDTOOLS <b>DNA EXTRACTION KIT</b>	Ref. 21.130M	Ref. 21.131	Ref. 21.132
SPEEDTOOLS TISSUE DNA EXTRACTION KIT	Ref. 21.135M	Ref. 21.136	Ref. 21.137
SPEEDTOOLS RNA VIRUS EXTRACTION KIT	Ref. 21.140M	Ref. 21.141	Ref. 21.142
SPEEDTOOLS FOOD DNA EXTRACTION KIT	Ref. 21.175M	Ref. 21.176	Ref. 21.177
SPEEDTOOLS PLANT DNA EXTRACTION KIT	Ref. 21.170M	Ref. 21.171	Ref. 21.172
SPEEDTOOLS TOTAL RNA EXTRACTION KIT	Ref. 21.210M	Ref. 21.211	Ref. 21.212
SPEEDTOOLS <b>PCR CLEAN-UP KIT</b>	Ref. 21.200M	Ref. 21.201	Ref. 21.202
SPEEDTOOLS PLASMID DNA PURIFICATION KIT	Ref. 21.220M	Ref. 21.221	Ref. 21.222

## 11. 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.biotoools.eu](http://www.biotoools.eu)), or by request to our Technical Dpt. ([technicalsupport@biotoools.eu](mailto:technicalsupport@biotoools.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.
8. The warranty provided herein and the data, specifications and descriptions of this kit appearing in BIOTOOLS published catalogues and product literacy is BIOTOOLS sole representations concerning the product and warranty. No other statements or representations, written or oral, by BIOTOOLS employees, agent or representatives, except written statements signed by a duly authorized officer of BIOTOOLS are authorized; they should not be relied upon by the customer and are not a part of the contract of sale or of this warranty.
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 ([technicalsupport@biotoools.eu](mailto:technicalsupport@biotoools.eu)).

### Manufactured by:

BIOTOOLS, Biotechnological & Medical Laboratories, S.A. has been evaluated and certified to accomplish ISO 9001 requirements for the following activities: Research and development of biotechnology products and manufacture of biotechnology and in vitro products. Valle de Tobalina – 52 – Nave 39, 28021 Madrid – Spain

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