PRODUCTS&SERVICES
Innovations for magnetic bioseparations

INSTRUCTIONS FOR USE

QuickPick™ SML Plant DNA

53002 • Plant DNA purification kit, 8 preps
53012 • Plant DNA purification kit, 24 preps
53022 • Plant DNA purification kit, 96 preps

TABLE OF CONTENTS:

INTRODUCTION

These are the instructions for use for the QuickPick™ SML Plant DNA purification kits. Please read the entire instructions carefully before starting the work. Also refer to QuicPick one magnet or QuicPick multiEight instructions for use.

The QuickPick™ SML Plant DNA purification kits provide fast and simple means of purifying genomic DNA from a variety of plants or their organelles. The technique does not require any organic solvents and eliminates the need for repeated centrifugation, vacuum filtration or column separation. The size of DNA purified using the QuickPick™ SML Plant DNA purification kits is typically at least 30 kbp. DNA fragments of this length denature completely during thermal cycling and can be used for downstream applications such as PCR amplifications, restriction enzyme digestions and sequencing.

The reagent volumes can be scaled up or down to be used with different sample amounts with the QuicPick manual tool.

Principle of the method

DNA in the plant tissue sample is released using Proteinase K solution and Lysis Buffer. Released DNA is bound to Magnetic Particles in the presence of Binding Buffer. Magnetic Particles with the bound DNA are washed three times with the Wash Buffer. The DNA is then eluted from the Magnetic Particles with the Elution Buffer.

SPECIFICATIONS

Table 1: Specifications for QuickPick™ SML Plant DNA purification kit.

Plant sample (leaf)	**Yields of DNA vs. sample amount[1]**		
	25 mg	**50 mg**	**100 mg**
Arabidopsis thaliana	Up to 1 µg	Up to 2 µg	Up to 4 µg
Lactuca sativa	Up to 1.5 µg	Up to 3 µg	Up to 6 µg
Hordeum vulgare	Up to 3 µg	Up to 6 µg	Up to 12 µg
Ocimum basilicum	Up to 2.5 µg	Up to 5 µg	Up to 10 µg
Typical purity[2]	≥ 1.7		
Size of purified DNA	≥ 30 kbp		

[1]DNA yield varies greatly between different sources of sample material.
[2]Ratio of absorbance at 260/280 nm is corrected with absorbance at 320 nm.

KIT CONTENTS

Reagents of the SML kits

Reagent:	**8 preps**	**24 preps**	**96 preps**
Plant DNA Magnetic Particles[1]	40 µl	170 µl	540 µl
Plant DNA Proteinase K solution	40 µl	250 µl	700 µl
Plant DNA Lysis Buffer	600 µl	3.2 ml	8.5 ml
Plant DNA Binding Buffer[2]	1 ml	4.25 ml	13.5 ml
Plant DNA Wash Buffer[2]	6 ml 22 ml	2 x 40 ml	
Plant DNA Elution Buffer	1 ml	7 ml	22 ml

[1]Reagents contain 0.02% NaN_3.

The reagents for QuickPick™ SML Plant DNA purification kits can also be bought separately:

Reagent:	**Volume**	**Product No:**
QuickPick™ XL Plant DNA Magnetic Particles[1]	2 ml	53100
QuickPick™ XL Plant DNA Proteinase K solution	2,8 ml	53200
QuickPick™ XL Plant DNA Lysis Buffer	32 ml	53400
QuickPick™ XL Plant DNA Binding Buffer[2]	50 ml	53300
QuickPick™ XL Plant DNA Wash Buffer[2]	300 ml	
QuickPick™ XL Plant DNA Elution Buffer	85 ml	53600

[1]Contains 0.02% NaN_3.

Scaling of sample amount

For both manual and automated protocols the sample amount can be scaled yielding to different number of preparations (Table 2) and reagent consumption. The reagent volumes are linearly dependent on the used sample amount. The reagent volumes for the manual purifications are shown Tables 3 and 4 (see Chapter 6 “Protocols for manual QuicPick one magnet and QuicPick multiEight tools”).

Table 2: The effect of sample amount to number of preparations and reagent volumes for the purifications with QuickPick Plant DNA SML kits.

Sample amount	**Number of preps**		
	53002[1]	**53012**	**53022**
25 mg	16	48	192
50 mg	8	24	96
100 mg	4	12	48

[1]For manual use only.

SAMPLE PREPARATION

DNA yield from plant tissues

The DNA content varies widely between different plant materials. For example, a tissue sample comprised of small cells will have a higher cell density, and therefore is likely to contain more nucleic acids than a sample of the same size which is comprised of larger cells. In addition, DNA content depends on the haploid genome size and the ploidy of the sample. For example, *Arabidopsis thaliana* has a small diploid genome and correspondingly lower DNA yields than wheat which has a large hexaploid genome.

When possible, it is preferable to harvest young plant material (e.g. expanding leaves or needles). Nucleic acid yields from young plant tissues are often higher than from old plant tissue, because young plant tissue generally contains more cells than the same amount of older plant tissue. In addition, young plant tissue of the same weight contains fewer metabolites (such as polyphenolics, polysaccharides and flavones) which can affect the performance of downstream applications.

Homogenization of plant material

Plant tissues should be homogenized before they are used as the sample in the purification protocol. Complete disruption of cell walls, plasma membranes, and organelle membranes is essential to release all the nucleic acids from the plant tissue. Insufficient homogenization of starting material will lead to low DNA yield. Cell wall properties vary widely between species and proper homogenization method should be applied to achieve complete disruption. The homogenization of plant tissue can be performed by mechanical grinding with different types of bead mills or with liquid nitrogen. Other homogenization methods can also be used.

Homogenization with liquid nitrogen using mortar and pestle

One of the most common homogenization methods involves freezing plant tissue sample in liquid nitrogen and grinding with a mortar and pestle.

1. Freeze plant tissue sample in liquid nitrogen immediately after harvesting. Do not let the sample to thaw at any time during homogenization.
2. Precool equipments by pouring liquid nitrogen into mortar and placing the pestles grinding end in the liquid nitrogen.
3. Place the frozen plant tissue sample in mortar and grind until fine whitish powder results.
4. Add liquid nitrogen as necessary but be careful not to spill the sample out of the mortar.
5. Using a precooled spatula transfer the powdered plant tissue sample into a precooled tube. Use several tubes for large samples to avoid thawing. Ensure all liquid nitrogen has evaporated before closing the tube(s).
6. If plant tissue sample is not processed immediately the tube should be kept on dry ice or liquid nitrogen or stored at -80oC, to prevent the sample from thawing after evaporation.
7. If plant tissue sample is processed immediately after homogenization, add the appropriate volume of Plant DNA Lysis Buffer and Proteinase K solution before the sample thaws.
8. Proceed with the purification protocol.

Homogenization using bead mill.

A bead mill homogenizes plant tissue samples by rapid agitation with tungsten carbide or steel beads. Homogenization is caused by the shearing and crushing action of the beads as they collide with the plant tissue sample. When using fresh plant leaf tissues, most samples can be homogenized in the presence of Lysis Buffer. Alternatively, homogenization of frozen plant material can be performed without Lysis Buffer if the beads and disruption vessel are precooled with liquid nitrogen. Plant tissue samples should be homogenized in the presence of either Lysis Buffer or liquid nitrogen to preserve the quality of the contained nucleic acids.

1. Weigh plant tissue samples into tubes.
2. Add appropriate volume of Plant DNA Lysis Buffer and 1 - 2 steel or Tungsten beads into each tube.
3. Close tubes tightly.
4. Homogenize for 1 - 2 minutes until the plant tissue samples seem homogenous.
5. Collect the beads away from the homogenates.
6. Pipette appropriate volume of Proteinase K solution into homogenates.
7. Proceed immediately with the purification protocol.

Homogenization using tissue grinder

A tissue grinder homogenizes plant tissue samples efficiently and helps in rapid preparation of the sample homogenates. When using fresh plant leaf tissues, most samples can be homogenized in the presence of Lysis Buffer. Alternatively, homogenization of frozen plant materials can be performed without Lysis Buffer if the disruption vessel is precooled with liquid nitrogen. Plant tissue samples should be homogenized in the presence of either Lysis Buffer or liquid nitrogen to preserve the quality of the contained nucleic acids.

1. Weigh plant tissue samples into tubes.
2. Add liquid nitrogen into the tubes but be careful not to spill the samples out of the tubes.
3. Homogenize the plant tissue samples for 1 - 2 minutes with the tissue grinder (for example Pellet Pestle or equivalent device) until fine whitish powder results.
4. Ensure all liquid nitrogen has evaporated before closing the tubes (Do not let the plant tissue samples thaw).
5. If plant tissue samples are not processed immediately the tubes should be kept on dry ice or liquid nitrogen or stored at -80°C, to prevent the samples from thawing after evaporation.
6. If plant tissue samples are processed immediately after homogenization, add appropriate volumes of Plant DNA Lysis Buffer and Proteinase K solution before the samples thaw.
7. Proceed with the purification protocol.

QUICPICK TIPS

The tips packed in bulk quantities in plastic bags are not sterile. Sterilize the tips in QuicPick Tip box by autoclaving.

PROTOCOLS FOR MANUAL QUICPICK one magnet AND multiEight TOOLS

QuicPick one magnet protocol

Notes

1. All solutions should be clear when used. If precipitates have formed, warm the solutions gently until the precipitates have dissolved.
2. Plant DNA Magnetic Particles should be mixed thoroughly just before pipetting. Vortexing of the Magnetic Particles is not recommended.
3. Repeat pipettors should not be used when dispensing Magnetic Particles.
4. If an RNA-free DNA preparation is required, add RNase solution into samples before starting the lysis step.
5. Water can also be used for elution.

Reagent volumes

Table 3: Reagent volumes for QuicPick 1purifications.

Reagent	**Reagent volume per preparation**		
Sample amount	**25 mg**	**50 mg**	**100 mg**
Lysis Buffer	37.5 µl	75 µl	150 µl
Proteinase K solution	2.5 µl	5 µl	10 µl
Binding Buffer	62.5 µl	125 µl	250 µl
Magnetic Particles	2.5 µl	5 µl	10 µl
Wash Buffer	3 x 125 µl	3 x 250 µl	3 x 500 µl
Elution Buffer	25 µl	50 µl	100 µl

Material required

1. Sterile 1.5 – 2.0 ml tubes.
2. Pipettes and sterile micropipettor tips.
3. QuicPick 1 tool and sterile QuicPick tips in a tip box.
4. Microcentrifuge.

Protocol

1. Number tubes from 1 to 6.

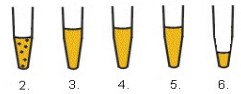
2. Prepare the plant tissue sample according to Chapter 4 “Sample preparation”. Add appropriate volumes of Lysis Buffer and Proteinase K solution into the plant tissue sample (see Table 3). Mix thoroughly by pulse-vortexing and lyse the sample for 15 - 30 minutes at 65°C.

1.

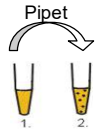
Incubate for 15 - 30 minutes at 65°C

3. During the lysis step, pipette QuickPick™ SML Plant DNA reagents (according to the Table 3) into tubes 2 - 6 as follows:

   Tube 2: Plant DNA Magnetic Particles and Plant DNA Binding Buffer
   Tube 3: Plant DNA Wash Buffer
   Tube 4: Plant DNA Wash Buffer
   Tube 5: Plant DNA Wash Buffer
   Tube 6: Plant DNA Elution Buffer

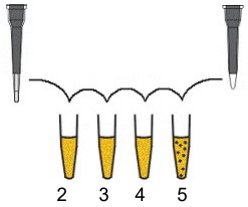


4. Remove tube 1 from 65°C. Centrifuge the tube for 5 minutes at 18,000 x *g*. Gently transfer the supernatant into tube 2 (Binding Buffer, Magnetic Particles). Mix tube 2 gently and incubate at room temperature for 2 - 10 minutes. Mix the suspension continuously during this step (use a tube rotator or mix manually).



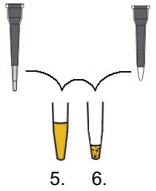
Incubate at room temperature with continuous mixing for 2 - 10 minutes

5. Pick up the QuicPick tip with the QuicPick 1. Collect the Magnetic Particles from tube 2 with QuicPick 1 and release them into tube 3 (Wash Buffer). Wash the Magnetic Particles by mixing the suspension gently for 10 - 20 seconds using the QuicPick tip. Note that the magnet has to be withdrawn at this point. To avoid the degradation of DNA only gentle mixing is recommended. Repeat the washing steps in tubes 4 and 5 (Wash Buffer).



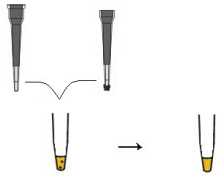
Washing steps

6. Collect the Magnetic Particles from tube 5 with the QuicPick 1 and release them into tube 6 (Elution Buffer). Mix tube 6 continuously and incubate at room temperature for 2 - 10 minutes (use a tube rotator or mix manually). During elution Magnetic Particles should disperse.



Incubate at room temperature with continuous mixing for 2 - 10 minutes

7. Collect the Magnetic Particles from tube 6 and discard them and the tip. The eluate in tube 6 containing the purified genomic DNA is ready to be used in downstream applications. If the purified DNA is not used on the same day, store at -20°C until use.



Collect the magnetic particles and discard them

QuicPick multiEight protocol

Notes

1. All solutions should be clear when used. If precipitates have formed, warm the solutions gently until the precipitates have dissolved.
2. Plant DNA Magnetic Particles should be mixed thoroughly just before pipetting. Vortexing of the Magnetic Particles is not recommended.
3. Repeat or 8-channel pipettors should not be used when dispensing Magnetic Particles.
4. If an RNA-free DNA preparation is required, add RNase solution into samples before starting the lysis step.
5. When using 96-well plates, the use of an orbital shaker is recommended. Adjust the speed to the highest possible level without causing liquid spill but still keep the Magnetic Particles in suspension.
6. Water can also be used for elution.

Reagent volumes

Table 4: Reagent volumes for QuicPick multiEight purifications

Reagent	**Reagent volume per preparation**		
Sample amount	**25 mg**	**50 mg**	**100 mg**
Lysis Buffer	37.5 µl	75 µl	150 µl
Proteinase K solution	2.5 µl	5 µl	10 µl
Binding Buffer	62.5 µl	125 µl	250 µl
Magnetic Particles	2.5 µl	5 µl	10 µl
Wash Buffer	3 x 125 µl	3 x 250 µl	3 x 500 µl
Elution Buffer	25 µl	50 µl	100 µl

Material required

1. Sterile U-bottom 96-well plates (for example Nunc 500 µl 96-well microplate or 1 ml 96 deep-well plate, also available from BN Products & Services Oy).
2. Pipettes and sterile micropipettor tips.
3. QuicPick multiEight tool and sterile QuicPick tips in a tipbox.
4. Microcentrifuge.
5. Orbital shaker for 96-well plates.

Table 5: Recommended plates for different sample amounts.

Sample amount (mg)	Recommended plate	BN Products & Services Product No.
25	Greiner 96-well microplate, 300 µl, sterile	M1-650261
50	Nunc 96-well microplate, 500 µl, sterile	M1-267245
100	Nunc 96 deep-well plate, 1 ml, sterile	M1-260251

Protocol

The following instructions are for 8 plant tissue samples. Samples are lysed in tubes and transferred into 96-well plates (U-bottom) where the rest of the protocol is carried out. The lysis step can also be performed in a thermal shaker using a suitable adapter for 96-well plates and if you have a centrifuge for these plates.

1. Prepare 8 plant tissue samples (number tubes from 1 to 8) according to Chapter 4 “Sample preparation”. Add appropriate volumes of the Lysis Buffer and Proteinase K solution into the tubes according to the Table 2. Mix the tubes well by pulse-vortexing and lyse samples for 15 - 30 minutes at 65°C.

Incubate at 65°C for 15 - 30 minutes

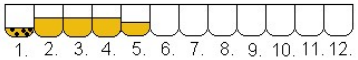
2. During the lysis step, pipette QuickPick™ SML Plant DNA reagents into 96-well plate columns 1 - 5 as follows (according to the Table 2).Column 1: Plant DNA Magnetic Particles and Plant DNA Binding Buffer

   Column 2: Plant DNA Wash Buffer

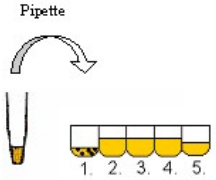
   Column 3: Plant DNA Wash Buffer

   Column 4: Plant DNA Wash Buffer

   Column 5: Plant DNA Elution Buffer



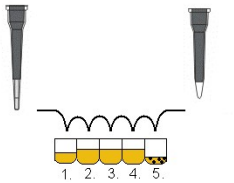
3. Remove the tubes from 65°C. Centrifuge tubes for 5 minutes at 18,000 x *g*. Gently transfer the supernatant from each tube into the respective wells of column 1 (Binding Buffer, Magnetic Particles) by mixing the lysed sample and Magnetic Particles carefully by pipetting up and down few times. Mix the 96-well plate on the orbital shaker for 2 - 10 minutes at room temperature. Make sure that the Magnetic Particles are in suspension during this step.



Incubate for 2 - 10 minutes at room temperature with continuous mixing

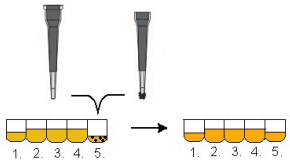
4. Pick up the QuicPick tips using QuicPick multiEight tool. Collect the Magnetic Particles from column 1 with QuicPick multiEight and release them into column 2 (Wash Buffer). Mix the suspensions gently for 10 – 20 seconds using the QuicPick tips. Note that the magnets have to be withdrawn at this point. To avoid the degradation of DNA only gentle mixing is recommended. Repeat the washing steps in columns 3 and 4 (Wash Buffer).

5. Collect the Magnetic Particles from column 4 with QuicPick multiEight and release them into column 5 (Elution Buffer). Mix the 96-well plate on the orbital shaker for 2 - 10 minutes at room temperature. Make sure that the Magnetic Particles are in a suspension during this step. During elution Magnetic Particles should disperse.



Incubate for 2 - 10 minutes at room temperature with continuous mixing

6. Collect the Magnetic Particles from column 5 and discard them and the tips. The eluates in column 5 contain the purified genomic DNA and are ready to be used in downstream applications. If the purified DNA is not used on the same day, store at -20°C until use.



Collect the Magnetic Particles and discard them

TROUBLESHOOTING GUIDE

Reagents

Low DNA yield	
Poor sample preparation Make sure that the sample is totally homogenized. Increase the homogenization time or try another homogenization method.	
	If liquid nitrogen is used: Don’t let the plant tissue samples to thaw during or after homogenization
	Cut the sample into small pieces before homogenization
Too small sample amount	Use larger sample amounts or smaller reagent amounts (see Chapter 3.2 “Scaling of sample amount”)
Too large sample amount	Use smaller sample amount. Too high sample amount interfere with the purification. For larger sample amounts use more reagents (see Chapter 3.2 “Scaling of sample amount”)
Insufficient lysis	Add correct volume or increase the volume of Proteinase K solution
	Use increased lysis time and/or improve mixing
	Make sure to mix plant tissue sample, Lysis Buffer and Proteinase K solution thoroughly by pulse-vortexing before starting the lysis incubation
	Ensure that the heating step is done at 65°C
	Use the appropriate heating adapter for the sample 96-well plate

Insufficient binding	Make sure the Magnetic Particles are in suspension during incubation
	Suspend the Magnetic Particles gently by pipetting up and down during binding step
	Increase the binding time
	Make sure the volume of the Binding Buffer correlates with the sample amount used
No shaking during incubations	Make sure the Magnetic Particles are in suspension during incubations
Insufficient washes	Increase the washing time in each Wash Buffer
	Use eluate as the sample and repeat the purification
Inappropriate elution buffer	DNA will only be eluted in the presence of low salt (e.g. 10 mM Tris-Cl, pH 8.5) or water. Check the pH and salt concentration of the elution buffer.
Insufficient elution	Increase the elution time
	Ensure that Magnetic Particles are in suspension during elution
	Continue elution until Magnetic Particles are uniformly dispersed
	Use heating during elution (max +65°C)
Magnetic Particles	Optimize the amount of Magnetic Particles
	Use only Plant DNA Magnetic Particles
	Do not freeze Magnetic Particles
	Make sure that Magnetic Particles are uniformly suspended before dispensing
Purified DNA too concentrated / too diluted	
Too small elution volume	Use more Elution Buffer to achieve optimal concentration
	Dilute final eluate by adding sufficient volume of Elution Buffer
Too large elution volume	Use less Elution Buffer to achieve optimal concentration

Manual Tools

Magnetic Particles are not collected from the suspension	
Magnet inside	Push the magnet out
No tip	Use correct tip
Sample too viscous	Make sure to use correct sample amounts and that homogenizing and lysis steps are adequately performed
	Decrease the amount of sample material
	Dilute the sample and use Lysis Buffer, Proteinase K solution and Binding Buffer in correct ratio
Visible Magnetic Particles in all vessels/wells	Increase the collecting time
Visible Magnetic Particles in Elution Buffer	Centrifuge the sample for 1 min with maximum speed
	Increase the collecting time
Particles not released from the tip	
Magnet out	Pull the magnet inside
No tip	Use tip
Sample amount too high	Make sure to use correct sample amounts and that the homogenization and lysis steps are adequately performed
	Decrease the amount of sample material
	Dilute the sample and use Lysis Buffer, Proteinase K solution and Binding Buffer in correct ratio
	Increase the suspension time and rub the QuicPick tip with Magnetic Particles against the vessel wall
Too small elution volume	Use larger volume

STORAGE AND STABILITY

The QuickPick™ SML Plant DNA purification kits should be stored at room temperature. Magnetic Particles should not be frozen.

WARNINGS AND LIMITATIONS

The QuickPick™ SML Plant DNA purification kit is intended for research use only, and is not intended for use in human diagnostic or therapeutic procedures. Standard methods for preventing contamination with DNases during preparation of DNA must be taken. Precautions should also be taken to avoid contamination of opened vials. Do not pipet by mouth.

Plant DNA reagent, Wash Buffer and Binding Buffer contain 0.02 % sodium azide (NaN_3) as a preservative. When in contact with acid or heavy metal ions, it forms a highly toxic gas. Preservatives such as NaN_3 are toxic if ingested. Do not pipet by mouth. Direct skin contact must be avoided. Appropriate precautions should be taken when handling these solutions.

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