USER GUIDE

for

Automated purification of DNA from Plant leaves and seeds with

KingFisher 96/ KingFisher mL instrument
and
MACHEREY-NAGEL NucleoMag 96 Plant kit

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Description

Purification of DNA from plant samples (e.g. green leaves or seeds) with MACHEREY-NAGEL´s NucleoMag 96 Plant kit can easily be automated using KingFisher® instruments (Thermo Fisher Scientific). The KingFisher platforms utilize patented technology where magnetic rods move particles through the processing steps. KingFisher 96 instrument operates on microplates and can process up to 96 samples per run, whereas KingFisher mL can handle 15 samples per run. With both instruments the volumes handled can be up to 1 ml.

Typically, DNA isolation from up to 50 mg of plant material using KingFisher instruments results up to 20 µg DNA. Generally, DNA yields vary according to sample type and storage conditions.

The protocol described here is designed for general use and can be modified according to customer individual needs using KingFisher® Software provided with the instrument.

Important notes

- See MACHEREY-NAGEL NucleoMag 96 Plant kit user manual for reagent storage, product use limitations, safety information etc.
- Resuspend magnetic beads (DNA Binding Beads) thoroughly before use.

Importing protocols from the web

KingFisher Software protocol for MACHEREY-NAGEL NucleoMag 96 Plant kit can be downloaded from the website (www.thermofisher.com/kingfisher). First you have to save the file **"NucleoMagPlantKF96"** to your computer.

1. Open KingFisher Software.
2. Select **Protocol** → **Import/Export data**.
3. Click **Read file**.
4. Select the database (***.KF2**) by browsing in the **Open** dialog and click **Open**.
5. Select the protocol(s) you wish to import from the *Protocols in file* list. Use the SHIFT key together with the mouse button to select protocols between two clicked protocols and the CTRL key to select only the clicked protocols.
6. Tick **Update existing** if you wish to overwrite the protocols with identical protocol name(s) in the target database.
7. Click **Import**.

If there are protocols with identical names and you have not ticked the **Update existing** tick box, you will be prompted to change the name of the protocol that is being imported:

- Type in a new name and click **OK**.
  - o **Note:** Check that the name of the protocol does not exceed 17 characters.
- You will receive a message stating whether the database updating procedure was successful or not.

Sending or running a protocol

To send a protocol to the instrument memory, or to run the protocol directly without saving it to the instrument memory:

1. Check that the instrument has been configured correctly and that the instrument is connected to the correct COM port.
2. Open the **Transfer protocol to instrument** dialog from **Instrument** → **Send Protocol to Instrument...**
3. Select the target instrument from the *Select instrument* table.
4. Select a protocol from the *Protocols for selected instrument* table. The protocols in that table are the available protocols in the database.
5. Select either of the following:

   a. Click **Send protocol** to transfer protocols to the instrument memory.
      - You can launch the protocol using the instrument keypad and display.

   b. Click **Execute protocol** to launch the protocol directly without transferring it to the instrument memory
      - The protocol will launch after validation.

KingFisher 96 protocol

Sample preparation

- KingFisher DNA protocol **NucleoMag PlantKF96** is designed to purify DNA from plant samples, e.g. leaves or seeds).

- Use Microtiter deepwell microplate (Catalog No. 95040450), KingFisher deepwell tip comb (Catalog No. 97002534) and KingFisher 96 KF plate (Catalog No. 97002540) with NucleoMag 96 Plant protocol.

- Add 500 µl of buffer MC1 and 10 µl of RNase A solution to sample. Perform lysis at 56°C for 30 min depending on samples. Remove debris by centrifugation to obtain a cleared lysate.
  Note! Lysis is done outside the instrument.

- Add sample and other reagents except Elution Buffer supplied by NucleoMag 96 Plant kit to KingFisher deepwell microplate according to table 1 and instructions below. Add the Elution Buffer to KingFisher 96 plate and start the NucleoMagPlantKF96 process.

KingFisher 96 process

Table 1 Pipetting instructions for KingFisher 96 and NucleoMag 96 Plant protocol.

Plate *	Plate	Content	Sample/ Reagent volume
A	1	Lysed Sample (cleared lysate)	400 µl
		Magnetic beads	30 µl
		Binding buffer MC2	400 µl
A	2	Wash buffer MC3	600 µl
A	3	Wash buffer MC4	600 µl
A	4	80 % ethanol	600 µl
A	5	Wash buffer MC5	600 µl
B	6	Elution buffer MB6	100 µl

* A= Thermo Deepwell plate, B=KingFisher 96 plate

1. Combine 400 µl of cleared lysate, 30 µl of resuspended Magnetic Beads and 400 µl Binding Buffer MC2 to plate **1**
2. Add 600 µl of Wash Buffer MC3 to plate **2**
3. Add 600 µl of Wash Buffer MC4 to plate **3**
4. Add 600 µl of 80% ethanol to plate **4**
5. Add 600 µl of Wash Buffer MC5 to plate **5**
6. Add 100 µl of Elution Buffer MC6 to plate **6**
7. Combine the tip comb and The KingFisher plate. See KingFisher 96 User manual.
8. Select the NucleoMag 96 Plant protocol using arrow keys and press START button OR execute protocol using KingFisher software
9. Load the plates according to protocol request and press START after every plate to confirm the action.
10. **Note!** Confirm that the plates are placed in correct orientation: A1 well to be pointed to upper right corner of the plate holder in turntable. A1 row of the plate is then always located in the inner circle of the turntable.
11. The purification protocol will start when the last plate is loaded and START button is pressed.
12. After the purification process is completed the plates are removed according to instructions shown in instrument screen. Press START after each plate removal to confirm the action.
13. When the last plate is removed text End_of _run will appear. Press STOP to complete the run.

Description of NucleoMag 96 Plant protocol with KingFisher 96

1. Samples are lysed with buffer MC1 and RNAse A for 30 min at 56°C. Following Lysis and a centrifugation step all further steps are done on the KingFisher 96 instrument.
2. Cleared lysate is incubated first with magnetic beads and Binding Buffer MC2 in plate 1 for 5 minutes. Magnetic bead/DNA complexes are formed.
3. Magnetic beads are washed with Wash Buffer MC3, MC4 and 80 % ethanol in plates 2, 3 and 4 respectively.
4. A final short wash step of magnetic beads with Wash buffer MC5 in plate 5 removes ethanol from previous wash buffers.
5. DNA is released to Elution Buffer MC6 in plate 6 for 10 minutes with heating
6. Beads are discarded into plate 4.

KingFisher mL protocol

Sample preparation

- KingFisher mL DNA protocol **NucleoMag PlantKFmL** is designed to purify DNA from plant samples e.g. leaves or seeds.
- Use KingFisher mL tubestrips and tip combs (Catalog No. 97002141) with **NucleoMagPlantKFmL** protocol.
- Add 500 µl of buffer MC1 and 10 µl of RNase A solution to sample. Perform lysis at 56°C for 30 min depending on samples. Remove debris by centrifugation to obtain a cleared lysate.
- Add sample and other reagents supplied by **NugleoMag 96 Plant** kit to KingFisher mL tubestrips according to table 2 and instructions below.

KingFisher mL process

Table 2 Pipetting instructions for KingFisher mL and NucleoMag 96 Plant protocol.

Tube	Content	Sample/ Reagent volume
A	Lysed sample (cleared lysate)	400 µl
	Magnetic beads	30 µl
	Binding buffer MC2	400 µl
B	Wash buffer MC3	600 µl
C	Wash buffer MC4	600 µl
D	80% Ethanol	600 µl
E	Elution buffer MC6	100 µl

1. Place an appropriate number of tube strips needed for the samples (one tube strip per sample) into removable tube strip tray.
2. Add 400 µl of cleared lysate, 30 µl of Magnetic Beads and 400 µl of Binding Buffer MC2 to tube strips **A**.
3. Add 600 µl of Wash Buffer MC3 and Wash Buffer MC4 to tube strip **B** and **C** respectively.
4. Add 600 µl of 80 % ethanol to tube strip **D**.
5. Add 100 µl of Elution Buffer MC6 to tube strip **E**.
6. Insert the tube strip tray to the instrument and insert the tip combs into the slots.
7. Close the front lid and start the process by selecting intended protocol **NugleoMagPlant_KFmL** using arrow keys and by pressing START button OR execute protocol using KingFisher software
8. Remove the tube strip tray from the KingFisher mL after program has completed.

Description of NucleoMag 96 Plant protocol with KingFisher mL

1. Samples are lysed with buffer MC1 and RNase A for 30 min at 56°C. Following lysis and a centrifugation step all further steps are done on the KingFisher mL instrument.
2. Cleared lysate is incubated first with magnetic beads and Binding Buffer MC2 in tube strip A.
3. Magnetic beads are washed with Wash Buffer MC3, MC4 and 80% ethanol in tube strips B, C and D respectively.
4. Magnetic beads are air-dried for 10 min.
5. Sample is released to Elution Buffer MC6 in tube strip E. Optionally: The sample is then moved manually to 1.5 ml reaction tube and incubated in a heat block for 10 minutes to release the DNA. The sample is then moved back to tube strip E.
6. Beads are discarded into tube strip D.

Trouble shooting

1. Low yield
   - ➢ Elution buffer volume insufficient
     - Beads pellet must be covered completely with elution buffer
   - ➢ Partial elution in Wash Buffer MC5 already
     - Keep the beads on the magnet while washing in Wash Buffer MC5. Do not resuspend beads in this buffer, and do not incubate beads in this buffer for more than 2 min, as this buffer is water-based and might elute the DNA already.

2. Low purity
   - ➢ Insufficient washing procedure
     - For KingFisher 96: Use only recommended Deep-well blocks (see KingFisher User Manual).
   - ➢ Carry-over of ethanol from 80% ethanol wash step
     - Increase time for washing step with buffer MC5 to 2 min. Alternatively add additional air dry step (5 min). Be sure to remove all of the 80% ethanol, as residual ethanol interferes with downstream applications.

3. If you have other questions related to chemistry see MACHEREY-NAGEL NucleoMag 96 Plant Kit User Manual for detailed troubleshooting instructions.

4. Any steps of the protocol (e.g. sample incubation and elution times) and the reagent volumes can be modified with KingFisher® software.

5. Tip comb was forgotten (KingFisher mL)
   - ➢ Clean the magnetic rods using a soft cloth or tissue paper soaked in mild detergent solution, soap or alcohol.

6. The processor is not working properly
   - ➢ Refer to Kingfisher mL or KingFisher 96 User Manual

Ordering Information

Product no.	Product Description
Thermo Fisher Scientific	
540 05 00	KingFisher 96, 110V-240V, Magnetic particle processor
24073430	KingFisher 96 head for Deep Well
97002534	KingFisher 96 tip comb for DW magnets (10 x 10 pcs/box)
97002540	KingFisher 96 KF plate (200 µl), 48 plates/box
95040450	Microtiter Deep Well 96 plate, V-bottom, Polypropylene
540 00 50	KingFisher mL, 110-240 V, Magnetic particle processor
97002131	KingFisher mL Combi 60 (tubes and tips for 60 samples)
97002141	KingFisher mL Combi 240 (tubes and tips for 240 samples)
97002111	KingFisher mL tip comb, 800 pcs
97002121	KingFisher mL tube, 900 pcs (20X45 pcs)
MACHEREY-NAGEL	
744 400.1	NucleoMag 96 Plant kit (1x96 preps)
744 400.4	NucleoMag 96 Plant kit (4x96 preps)
744 400.24	NucleoMag 96 Plant kit (24x96 preps)

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