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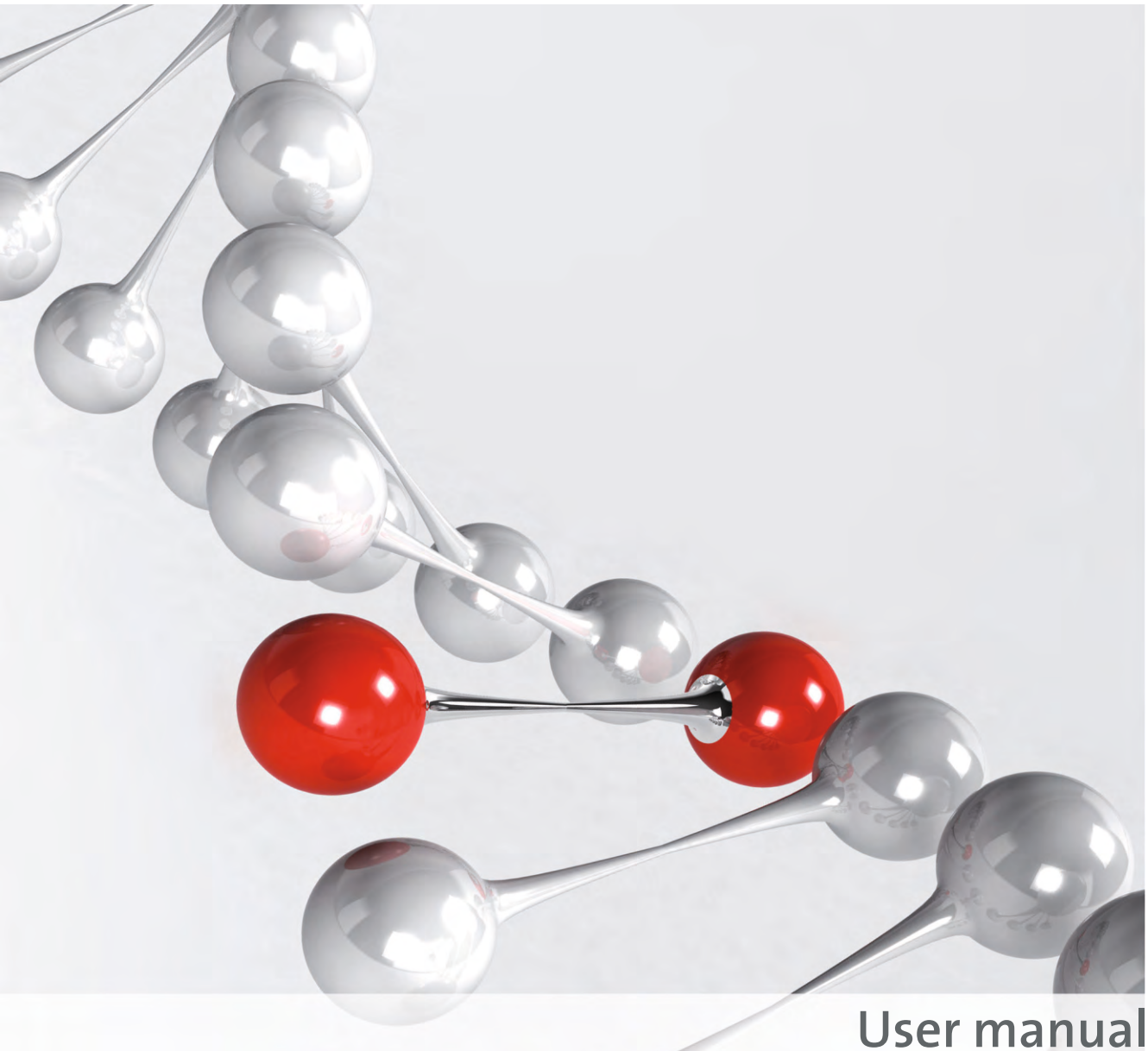


User manual
InviMag® Plant DNA Mini Kit/ KF96

for use on KingFisher™ 96 and KingFisher™ Flex, Thermo Fisher Scientific

for automated purification of genomic DNA from up to 100 mg plant material and food of vegetable origin with magnetic beads

REF 7437300X0

STRATEC Molecular GmbH, D-13125 Berlin

Instruction for InviMag® Plant DNA Mini Kit/ KF96

The **InviMag® Plant DNA Mini Kit/ KF96** is the ideal tool for isolation and purification of DNA from up to 100 mg plant material or food from plant origin with the patented InviMag® technology using a KF96 / KFflex96 instrument.

The kit is neither validated for the isolation of genomic DNA from cultured or isolated cells, from blood, stool samples, swabs, dried blood stains or cell free body fluids, like cerebrospinal fluid, synovial fluid and urine nor from bacteria, fungi, parasites or purification of RNA.

Table of content

Kit contents of InviMag® Plant DNA Mini Kit/ KF96

	96 extractions	5 x 96 extractions
Catalogue No.	7437300100	7437300200
Lysis Buffer P	50 ml	210 ml
Proteinase K working solution	2 x 1.5 ml	10.5 ml
Binding Buffer A	9 ml (final volume 30 ml)	36 ml (final volume 120 ml)
SNAP Solution	2 x 1.1 ml	10.5 ml
Wash Buffer I	80 ml (final volume 160 ml)	3 x 80 ml (final volume 3 x 160 ml)
Wash Buffer II	60 ml (final volume 200 ml)	4 x 60 ml (final volume 4 x 200 ml)
Elution Buffer	15 ml	60 ml
2.0 ml Deep Well Plate	4 pieces	20 pieces
KF96 Tip Comb for DW magnets	1 piece	5 pieces
200 µl Elution Plate*	2 pieces	10 pieces
Prefilter Plate I	1 piece	5 pieces
Sealing Foils	2	10
Manual	1	1
Initial steps	Add 1.5 ml distilled water to each **Proteinase K** tube, mix thoroughly until completely dissolving Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer I** Add 140 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and always keep the bottles firmly closed Add 21 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.	Dilute **Proteinase K** in 10.5 ml of ddH_2O, mix thoroughly until completely dissolving Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer I** Add 140 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and always keep the bottles firmly closed Add 84 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.

* Elution and Tip Plate are identically. Use one provided Elution Plate as a Tip Plate.

Kit contents of InviMag® Plant DNA Mini Kit/ KF96 w/o plastic

	96 extractions	5 x 96 extractions
Catalogue No.	7437300150	7437300250
Lysis Buffer P	50 ml	210 ml
Proteinase K working solution	2 x 1.5 ml	10.5 ml
Binding Buffer A	9 ml (final volume 30 ml)	36 ml (final volume 120 ml)
SNAP Solution	2x 1.1 ml	10.5 ml
Wash Buffer I	80 ml (final volume 160 ml)	3 x 80 ml (final volume 3 x 160 ml)
Wash Buffer II	60 ml (final volume 200 ml)	4 x 60 ml (final volume 4 x 200 ml)
Elution Buffer	15 ml	60 ml
Sealing Foils	2	10
Manual	1	1
Prefilter Plate I	1	5
Initial steps	Add 1.5 ml distilled water to each **Proteinase K** tube, mix thoroughly until completely dissolving Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer I** Add 140 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and always keep the bottles firmly closed Add 21 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.	Dilute **Proteinase K** in 10.5 ml of ddH_2O, mix thoroughly until completely dissolving Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer I** Add 140 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and always keep the bottles firmly closed Add 84 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.
Plastic to be supplied by user (see order information)		
2.0 ml Deep Well Plate	4	20
KF 96 Tip Comb for DW magnets	1	5
200 µl Elution Plate*	2	10

* Elution and Tip Plate are identically. Use one provided Elution Plate as a Tip Plate.

Symbols

 Manufacturer

 Lot number

 Catalogue number

 Expiry date

 Consult operating instructions

 Temperature limitation

 Do not reuse

Storage

All buffers and kit contents of the **InviMag® Plant DNA Mini Kit/ KF96,** except **dissolved Proteinase K** should be stored at room temperature and are stable for at least 12 months.

Room temperature (RT) is defined as range from 15-30°C.

Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by warming carefully (up to 30°C).

Proteinase K: Dissolved Proteinase K must be stored at 2 - 8 °C for up to two months. For longer storage –20 °C is recommended, freeze-thaw once only. So the dissolved Proteinase K is stable as indicated on the kit package.

Wash Buffers charged with ethanol should be appropriately sealed and stored at room temperature.

Binding Buffer charged with isopropanol should be appropriately sealed and stored at room temperature.

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **InviMag® Plant DNA Mini Kit/ KF96** for applications as described in this manual. Purchaser must determine the suitability of the product for its particular use. Should any product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the product free of charge.

STRATEC Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.

In accordance with STRATEC Molecular's EN ISO 9001 and EN ISO 13485 certified Quality Management System the performance of all components of the **InviMag® Plant DNA Mini Kit/ KF96** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **InviMag® Plant DNA Mini Kit/ KF96** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:

from Germany +49-(0)30-9489-2901/ 2910
from abroad +49-(0)30-9489-2907 or contact your local distributor.

Intended use

The **InviMag® Plant DNA Mini Kit/ KF96** is designed for a fully automated preparation of genomic DNA from up to 100 mg plant or food (plant origin) material using the patented **InviMag® technology** in combination with a KF96/ KFflex96 instrument.

The whole process is based on a patented bead technology, used for isolation of genomic DNA by binding the nucleic acid onto magnetic particles in absence of chaotropic buffer components.

For reproducible and high yields appropriate sample storage is essential.

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONALS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of RNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in your results, adequate controls for downstream applications should be used.

Product use limitation

The kit is neither suitable for the isolation of DNA from blood, serum or plasma, bacteria, fungi or viruses, nor for isolation and purification of RNA.

The included chemicals are only useable once.

Differing of starting material or flow trace may lead to inoperability; therefore neither a warranty nor guarantee in this case will be given, neither implied nor express.

The user is responsible to validate the performance of the STRATEC Molecular product for any particular use. STRATEC Molecular does not provide for validation of performance characteristics of the product with respect to specific applications. STRATEC Molecular products may be used e.g.in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA' 88 regulations in the U.S. or equivalents in other countries.

All products sold by STRATEC Molecular are subject to extensive quality control procedures (according to EN ISO 9001 and EN ISO 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.

The chemicals and the plastic parts are for laboratory use only; they must be stored in the laboratory and must not be used for purposes other than intended.

The product with its contents is unfit for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **InviMag® Plant DNA Mini Kit/ KF96** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, the liquid waste has to be considered infectious and be handled and discarded accordingly to local safety regulations.

European Community risk and safety phrases for the components of the **InviMag® Plant DNA Mini Kit/ KF96** to which they apply, are listed below as follows:

Lysis Buffer P

danger
H319 P305-351-338

Proteinase K:

danger
H315-319-334-335 P280-305-351-338-310-405

Wash Buffer I

warning
H302-312-332-412 EUH032 P273

H315:	Causes skin irritation.
H319:	Causes serious eye irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
H302:	Harmful if swallowed.
H312:	Harmful in contact with skin.
H332:	Harmful if inhaled.
H412:	Harmful to aquatic life with long lasting effects.
EUH032:	Contact with acids liberates very toxic gas.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338:	If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses if present. Continue rinsing.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.
P273:	Avoid release to the environment.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: **1 – 352 – 323 – 3500**
inside of USA : **1 – 800 – 535 – 5053**

Product characteristic of the InviMag® Plant DNA Mini Kit/ KF96

Starting material	Yield	Time	Ratio
up to 100 mg plant material	5 - 25 µg; depends on the kind and amount of starting material	about 30 min (without lysis)	A_{260}:A_{280} 1.8-2.2

The **InviMag® Plant DNA Mini Kit/ KF96** is designed for a semi-automated preparation of genomic DNA from up to 100 mg of plant material using magnetic beads and the KF96 or KFflex96 workstation. The isolation process is based on the patented **InviMag®** technology.

The DNA isolation process relies on the interaction of nucleic acids with coated magnetic particles at adapted buffer conditions. The KF96 / KFflex96 instrument performs all purification steps of the DNA purification procedure automatically, except the plant sample preparation, lysis and initial loading of the system. Sample cross-contamination and reagent cross-over is effectively eliminated by the provided assay file.

The KingFisher® instrument uses magnetic rods to transport the DNA bound to magnetic particles through the various assay phases like binding, washing, drying and elution. The volume of buffers and other liquids required for DNA isolation is reduced to a minimum.

To achieve optimal lysis conditions and high yields, the plant samples are first mechanically disrupted followed by a lysis step in an optimized buffer system at elevated temperature. After lysis, a binding step is performed in which the DNA is bound to the magnetic particles followed by several washing steps before the pure DNA is finally eluted.

The purified and high quality DNA can be stored at -20°C for subsequent use and is ready-to-use for subsequent downstream applications like:

- PCR*
- Genotyping
- Restriction digestion

No toxic or hazardous chemicals are used.

For the isolation of DNA from single plant samples STRATEC Molecular offers the **Invisorb® Spin Plant Mini Kit**, as well as 96 well kits for use in a centrifuge.

For further information please contact +49 (0) 30 9489 2901 or 2910 in Germany and +49 (0) 30 9489 2907 from foreign countries or ask your local distributor.

Sampling and storage of starting material

Harvested plant samples can be stored at room temperature for up to 2–3 hours. For short-term storage (up to one week) samples may be stored at 2-8°C. For long-term storage, we recommend freezing samples at -20°C or -80°C. Multiple thawing and freezing cycles before isolating the DNA should be avoided because this can lead to degraded DNA and reduced yields.

STRATEC Molecular will be released of its responsibilities if other sample materials than described in the Intended Use are processed or if the sample preparation protocols are changed or modified.

Principle and Procedure

Lysis

Samples are disrupted by using a mixer mill, bead mill or by mortar and pistil in combination with liquid nitrogen. Afterwards, the disrupted material is lysed at denaturing non-chaotropic conditions at elevated temperatures in presence of **Lysis Buffer P** and **Proteinase K**. Because unlysed material has to be removed after lysis by filtration, the lysis procedure has to be performed externally. The filtration step is required, because in most cases a centrifugation step will not be able to remove very small debris, especially if a mortar and pistil was used for disrupting.

Binding of the DNA

After addition of **Binding Buffer A** and **SNAP Solution** to the lysate, optimal binding conditions are adjusted and the genomic DNA is bound to the magnetic particles.

Removing residual contaminants

Contaminants are efficiently removed using **Wash Buffer I** and **Wash Buffer II**, while the DNA remains bound to the magnetic beads.

Elution

The DNA is finally eluted in **Elution Buffer**. The eluted DNA is ready-to-use in different subsequent downstream applications like:

- PCR*, RAPD, AFLP analysis
- microsatellite analysis
- genotyping
- enzymatic restriction digestion

Yield and quality of genomic DNA

The amount of purified DNA derived by the **InviMag® Plant DNA Mini Kit /KF96** procedure from plant materials depends on the sample source, transport conditions, storage and sample age.
The overall yield and quality of the isolated genomic DNA is suitable for any detection system.

*The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

Important points before starting a protocol

Immediately upon receipt of the product, inspect the product and its components as well as the package for any apparent damages, correct quantities and quality. If there are any unconformities you have to notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety Information" (see page 7). Do not use damaged kit components, since their use may lead to poor kit performance.

- Change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard contaminated gloves immediately.
- Do not combine components of different kits, unless the lot numbers are identical.
- Avoid microbial contamination of the kit reagents.
- This kit should only be used by trained personnel.

Preparing reagents and buffers

Before starting a run, bring all reagents to room temperature. Gently mix and redissolve any precipitates by warming up to 30°C. Swirl gently to avoid foaming.
Lysis Buffer P and **Elution Buffer** are ready-to-use.
Add the required amount of ddH_2O to the reaction tube containing the **Proteinase K**. Vortex for 5 s

1x 96 DNA-extractions
Add 21 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.
Dilute **Proteinase K** by addition of 1.5 ml of ddH_2O, mix thoroughly until completely dissolving
Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer I**
Add 140 ml of 96-100% ethanol to the bottle **Wash Buffer II**.
Mix thoroughly and always keep the bottle firmly closed
5x 96 DNA-extractions
Add 84 ml 99.7% Isopropanol to the **Binding Buffer A.** Mix by intensive shaking by inverting for 1 min. Shortly before use mix by inverting several times.
Dilute **Proteinase K** by addition of 10.5 ml of ddH_2O, mix thoroughly until completely dissolving
Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer I**
Add 140ml of 96-100% ethanol to the bottle **Wash Buffer II**
Mix thoroughly and always keep the bottle firmly closed

Reagents and equipment to be supplied by user

- Measuring cylinder (250 ml)
- Pipette and pipette tips
- Disposable gloves
- ddH_2O
- Vortexer
- 96-100% ethanol
- Isopropanol

*The **InviMag® Plant DNA Mini Kit/ KF96** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth**

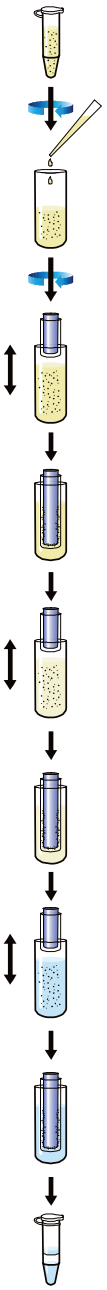
*** Possible suppliers for Isopropanol:**

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order Nr. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

Scheme of the InviMag® Plant DNA Mini Kit/ KF96



Transfer the crushed plant material into 1.5 ml reaction tubes (not provided) or a 96 DWP (not provided) and add 400 µl **Lysis Buffer P** and 20 µl **Proteinase K**.

Incubate the lysis mixture for 30 min at 60°C while continuously shaking.

After lysis, a filtration step of the lysis mixture into the Binding Plate, prefilled with 200 µl **Binding Buffer A** *(follow preparing instructions)* and 20 µl **SNAP Solution**, is performed.

DNA binds to magnetic particles

Magnetic separation

Washing of the particle fixed genomic DNA with 800 µl **Wash Buffer I** and twice with 800 µl **Wash Buffer II**

Magnetic separation and drying step

Elution of genomic DNA with 100 µl **Elution Buffer**

Magnetic Separation and bead removal

Pure DNA

Lysis Procedures

Protocol: Isolation of genomic DNA from up to 100 mg of plant material

Please read the instructions carefully and conduct the prepared procedure.

Homogenization of the starting material

Homogenize up to 100 mg of plant material by use of a pestle and mortar in combination with liquid nitrogen. Commercially available equipment for homogenization (bead mill, etc.) can be used too.

Transfer the homogenized starting material either into 1.5 ml reaction tubes (not provided) or use a 96 DWP (not provided) and add 400 µl of Lysis Buffer P and 20 µl of Proteinase K to each sample. Incubate the mixtures at 60°C for 30 min while continuously shaking.

During lysis, prefill all plates with the required buffers and appropriate volumes (see "Starting a run", page 12). After lysis, set up the Prefilter plate I onto the Binding Plate (2.0 ml Deep Well Plate) and transfer the lysis mixtures into the filter plate. Incubate for 2-3 min at room temperature or until all lysates have passed the filter plate. The lysed samples have now been transferred into the Binding Plate.

Remove the filter plate and start the run on the KF instrument (see below).

Important: *The kit will also co-purify RNA beside DNA. For the elimination of RNA (if required) add 20 µl RNase A (10 mg/ml) to the Lysis Buffer P prior before starting the lysis procedure.*

Starting a Run on a KF96 / KFflex96 instrument

Attention: *Please be aware, that you have to prepare the **Binding Buffer A** – see instruction page: 10*

Note: *Before starting the purification process with the KingFisher instrument please carefully read the manufacturer´s manual! Resuspend/Vortex the magnetic particles (SNAP Solution) thoroughly before use!*

1. Switch on the KingFisher instrument
2. Prefill all required plates as described below:

Tip Plate:	Place the KF96 Tip Comb for DW magnets on a Tip Plate (Use one provided Elution Plate as Tip Plate. These are identical.)
Binding Plate:	200 µl **Binding Buffer A**, 20 µl **SNAP Solution** to a 2 ml Deep Well Plate
Washing_Plate_1:	Add 800 µl **Wash Buffer I** to a 2 ml Deep Well Plate
Washing_Plate_2:	Add 800 µl **Wash Buffer II** to a 2 ml Deep Well Plate
Washing_Plate_3:	Add 800 µl **Wash Buffer II** to a 2 ml Deep Well Plate
Elution Plate:	Add 100 µl **Elution Buffer** to the 200 µl Elution Plate

3. Choose either the assay file **"InviMag_Plant_KF96"** (KF96 assay file) or **"InviMag_Plant_KFflex96"** (KFflex96 assay file) on the display of the corresponding KingFisher instrument and press the "START" button.
4. Insert the prefilled plates onto the right positions of the KingFisher instrument by following the specifications shown on the instrument display and confirm every loading step with the "START" button. When all prefilled plates are loaded press the "START" button to initialize the assay file. The assay file will start at the Binding Step. From this point, the instrument will continue with the purification process without any further user interaction.

The following extraction steps run automatically on the KingFisher™ System!

1. **Binding of the DNA**

   Automatically sample mixing for 5 min. SNAPs separation. Transfer of the SNAPs to Washing Plate 1.

2. **First Washing**

   Automatically sample mixing for 1.5 min. SNAPs separation. Transfer of the SNAPs to Washing Plate 2.

3. **Second Washing**

   Automatically sample mixing for 1 min. SNAPs separation. Transfer of the SNAPs to Washing Plate 3.

4. **Third Washing**

   Automatically sample mixing for 1 min. SNAPs separation.

5. **Drying**

   Drying of SNAPs outside Washing Plate 3 for 5 minutes. Transfer of the SNAPs to the Elution Plate.

6. **Elution of the DNA**

   Incubation of SNAPs for 10 minutes at 65°C while continuously mixing. SNAPs separation. Removal of SNAPs into Washing Plate 3 (disposal).

Important Notes:

1. *After finishing the extraction protocol, the Elution Plate contains the extracted DNA. Store the DNA at adequate conditions. For long-term storage we recommend to store the DNA at -20°C.*
2. *If the extracted DNA contains carryover from magnetic particles, transfer the DNA into a new 1.5 ml reaction tube and centrifuge at maximum speed for 1 minute. Transfer the clear supernatant (containing the DNA) into a new tube.*

The eluted DNA is ready-to-use in different downstream applications. The eluted DNA can be stored for several weeks at 4-8°C or stored at -20°C for long-term storage.

For self-programming of the KF96 / KFflex96 instrument

Reagent info

Tip Plate			KingFisher 96 KF plate
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
-	-	-	-

Binding Plate			Microtiter DW 96 plate
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Crushed sample material in LysisBuffer P	420	-	Sample
Binding Buffer A	200	-	Reagent
SNAP Solution	20	-	Reagent

Washing Plate 1			Microtiter DW 96 plate
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer I	800	-	Reagent

Washing Plate 2			Microtiter DW 96 plate
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer II	800	-	Reagent

Washing Plate 3			Microtiter DW 96 plate
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Wash Buffer II	800	-	Reagent

Elution Plate			KingFisher 96 KF plate
Name	**Well volume [µl]**	**Total reagent volume [µl]**	**Type**
Elution Buffer	100	-	Reagent

Dispensed reagents

The protocol does not contain dispensed reagents

Steps data

Tip1		96 DW tip comb	
	Pick-Up	Tip Plate	
	Binding Step	Binding Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:15, Fast
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	10
	Washing Step 1	Washing Plate 1	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:30, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	5
	Washing Step 2	Washing Plate 2	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	5
	Washing Step 3	Washing Plate 3	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:01:00, Fast
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	3
		Collect time [s]	5
	Drying	Washing Plate 3	
		Dry time	00:05:00
		Tip position	Outside well / tube
	Elution	Elution Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Medium
	Mixing / heating:	Mixing time, speed	00:10:00, Slow
		Heating temperature [°C]	65
		Preheat	Yes
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	10
	Bead Removal	Washing Plate 3	
		Release time, speed	00:00:30, Fast
	Leave	Tip Plate	

Troubleshooting

Problem	Probable cause	Comments and suggestions
low amount of extracted DNA	insufficient lysis	increase lyses time, but prevent too long lyses time because this also decreases the yield Reduce amount of starting material
	incomplete elution	increase volume of **Elution Buffer /** increase time of elution step
	low amount of **SNAP Solution**	mix **SNAP Solution** thoroughly before addition
low concentration of extracted DNA	too much **Elution Buffer**	elute the DNA with a lower volume of **Elution Buffer** (don´t use less than 100 µl)
	incorrect storage of starting material	ensure that storage of starting material is correct avoid repeated freezing and thawing cycles of the material
degraded / sheared DNA	incorrect storage of starting material	ensure that the storage of starting material was correct avoid multiple freezing and thawing cycles of the sample material
	old material	ensure that the starting material is fresh or stored at appropriate conditions (long-term storage at -20°C) old material often contains degraded DNA
DNA does not perform well in downstream-applications (e.g. real-time PCR or PCR)	ethanol carryover during elution	increase drying time for evaporation of ethanol
	salt carryover during elution	check the **Wash Buffers** for salt precipitates. If any precipitates are visible, solve them by carefully warming up to 30°C ensure that the **Wash Buffers** are equilibrated at room temperature
low A_{260}:A_{280} ratio from UV measurement, eluted DNA is brown colored	small part of the magnetic particles are left in the elution	centrifuge at full speed for 1 min and transfer supernatant to a new tube

Appendix

KingFisher™ BindIt Software 3.2 or higher versions

BindIt software 3.2 or higher versions were and may be used to create assay files for the KFmL, KF96/KFflex96 or KF-Duo instruments. The provided assay file(s) can either be transferred onto the corresponding workstation(s) or be started directly from within the BindIt software after assay import. Please keep in mind, that assay(s) run from within the BindIt software are not stored in the workstation memory.

Important: *Be advised that BindIt SW 3.2 or higher versions use a new unique file extension. Therefore, it is not possible to import assay files created with BindIt 3.2 or higher versions into older BindIt software versions! Please ask your local Thermo Scientific distributor for a software update.*

Note*:* *When creating assay files for usage with KingFisher™ instruments in combination with Microtiter Deep Well plates (e.g. Thermo Electron), it is essential to use the KingFisher™ software 3.2 or higher versions for assay development because this software version includes the correct adjustments for the microtiter plate. It is highly recommended to use Thermo Microtiter Deep Well plates with KF96 / KFflex96 / KF-Duo workstations to ensure the best purification result.*

Minimum system requirements for BindIt Software 3.2 or higher versions

PC requirements	
Supported operating systems	MS Windows XP Pro with SP3, Windows Vista SP2, Windows 7
Disk space	500 MB free disk space
Processor	Intel Pentium ≥ 1 GHz
Memory	1 GB RAM
Serial ports available	1 (for KFmL connection)
USB ports available	1 (for KF96 / KFflex96 / KFDuo connection)
Pointing device	Mouse or equivalent is required
CD-ROM drive	1
Monitor / color settings	XVGA monitor with at least 1024x768 resolution and at least a 16-bit color environment

If the actual Windows Service Packs are not installed on the corresponding lab computer, they can be downloaded from the Microsoft web pages: http://www.microsoft.com/

General notes on handling DNA

Nature of DNA

The length and delicate physical nature of DNA requires careful handling to avoid damage due to shearing and enzymatic degradation. Other conditions that affect the integrity and stability of DNA include acidic and alkaline environments, high temperature, and UV irradiation. Careful isolation and handling of high molecular weight DNA is therefore required to ensure compatibility with various downstream applications. Damaged DNA may perform poorly in applications such as genomic Southern Blotting or long-template PCR.

Storage of DNA

A working stock of DNA can be stored at 2-8°C for several weeks. For long-term storage DNA should be stored at -20°C, but storing at -20°C may cause shearing, particularly if the DNA is exposed to repeated freezing and thawing cycles.

Note that the solution, in which the nucleic acid is eluted in, will affect it's stability during storage. Pure water lacks buffering capacity and an acidic pH may lead to acid hydrolysis. Tris or Tris-EDTA buffer contains sufficient buffering capacity to prevent acid hydrolysis.

Drying, dissolving and pipetting DNA

Avoid overdrying of genomic DNA after ethanol precipitation. We highly recommend to air dry than to use a vacuum, although vacuum drying can be used with caution.

Avoid vigorous pipetting. Pipetting of genomic DNA through small tip openings can cause shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings designed for pipetting genomic DNA.

DNA yield

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

Ordering information

InviMag® Plant DNA Mini Kit /KF96	7437300100	1 x 96 preps
InviMag® Plant DNA Mini Kit /KF96	7437300200	5 x 96 preps
InviMag® Plant DNA Mini Kit/ KFmL	2437110200	75 purifications
InviMag® Plant DNA Mini Kit/ KFmL	2437110400	300 purifications
Invisorb® DNA Plant HTS 96 Kit/ C	7037300200	2 x 96 preps
Invisorb® DNA Plant HTS 96 Kit/ C	7037300300	4 x 96 preps
Invisorb® DNA Plant HTS 96 Kit/ C	7037300400	24 x 96 preps
Invisorb® Spin Plant Mini Kit	1037100200	50 purifications
Invisorb® Spin Plant Mini Kit	1037100300	250 purifications

Possible suppliers for Isopropanol:

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

Ordering information (KingFisher™ 96 and consumables)

Cat.no	Description
5400500	KingFisher 96, magnetic particle processor,100-240V,50/60Hz (including one magnetic head)
24073430	KingFisher 96 head for Deep Well Plates
97002514	KingFisher 96 tip comb for a PCR magnet head / plates, 8 x 10 pcs / box
97002524	KingFisher 96 tip comb for KF magnets / plates, 10 x 10 pcs / box
97002534	KingFisher 96 tip comb for DWP magnets / DWP 10 x 10 pcs / box
97002540	KingFisher 96 KF plate (200 µl) 48 plates / box
95040450	Microtiter deep well 96 plate (2 ml), 50 plates / box

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