

User manual

InviMag[®] Tissue DNA Mini Kit/ KF96

for use on KingFisher[™] 96 and KingFisher[™] Flex, Thermo Fisher
for automated purification of genomic DNA from up to 20 mg of tissue sample, mouse
or rodent tail and eucaryotic cell pellets with magnetic beads



REF 7432300x00



STRATEC Molecular GmbH, D-13125 Berlin

Instruction for InviMag® Tissue DNA Mini Kit/ KF96

The **InviMag® Tissue DNA Mini Kit/ KF96** combines the advantages of the innovative Invisorb® technology with easy handling of magnetic particles for a very efficient and reliable isolation of nucleic acids with a high purity.

The DNA-binding magnetic particles are characterized by a high surface area, a uniform size distribution, and good suspension stability and are therefore highly suitable for high throughput processing.

The **InviMag® Tissue DNA Mini Kit/ KF96** for isolation and purification of total (genomic and mitochondrial) DNA from tissue samples, biopsy samples, frozen sections, rodent tails, insects and eucaryotic cell pellets in a 96 well format is designed for an optimal use on the KingFisher™ 96 or Flex workstation from Thermo Scientific. The interplay of the DNA extraction and purification chemistry provided by the **InviMag® Tissue DNA Mini Kit/ KF96** with the KingFisher™ machine was intensely tested and validated.

Due to the high purity, the isolated DNA is ready to use for *in vitro* diagnostic analysis and for a broad panel of downstream applications or can be stored at -20°C for subsequent use.

The kit is neither suitable for isolation of DNA from blood, stool sample, swabs, dried blood stains or cell free body fluids, like cerebrospinal fluid, synovial fluid and urine nor from bacteria, plant material, fungi, parasites or the purification of RNA.

The application of the kit for isolation and purification of viral DNA has not been evaluated.



Compliance with EU Directive 98/79/EC on *in vitro* medical devices.

Not for *in vitro* diagnostic use in countries where the EU Directive 98/79/EC on *in vitro* medical devices is not recognized.

Trademarks: InviMag®, Invisorb®. Registered marks, trademarks, etc. used in this document, even when not specifically marked as such, are not to be considered unprotected by law.

The Invisorb® technology is covered by patents and patent applications: US 6,110,363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

InviMag® and Invisorb® are registered trademarks of STRATEC Biomedical AG.

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

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Kit contents of InviMag[®] Tissue DNA Mini Kit/ KF96

	1 x 96 extractions	5 x 96 extractions
Catalogue No.	7432300100	7432300200
Lysis Buffer G	50 ml	220 ml
Binding Buffer A	9 ml (final volume 30 ml)	36 ml (final volume 120 ml)
Proteinase K (working solution)	3 x 1 ml	13 ml
MAP Solution A	2 x 1.2 ml	10.5 ml*
Wash Buffer	2 x 45 ml final volume 2 x 150 ml	6 x 60 ml final volume 6 x 200 ml
Elution Buffer	30 ml	120 ml
2.0 ml Deep Well Plate	4	20
KF 96 Tip Comb for DW magnets	1	5
200 µl Elution Plate*	2	2 x 5
1.5 ml Receiver Tubes	2 x 50	10 x 50
Sealing Foils	2	10
Manual	1	1
Initial steps	<p>Add 21 ml of 99.7% Isopropanol to the Binding Buffer A. Mix by intensive shaking by inverting for 1 min.</p> <p>Dilute Proteinase K by addition of 1 ml of ddH₂O, mix thoroughly until completely dissolving</p> <p>Add 105 ml of 96-100% ethanol to the bottle Wash Buffer, mix thoroughly and keep the bottle always firmly closed!</p>	<p>Add 84 ml of 99.7% Isopropanol to the Binding Buffer A. Mix by intensive shaking by inverting for 1 min.</p> <p>Dilute Proteinase K by addition of 13 ml of ddH₂O, mix thoroughly until completely dissolving</p> <p>Add 140 ml of 96-100% ethanol to the bottle Wash Buffer, mix thoroughly and keep the bottle always firmly closed!</p> <p>*Divide the MAP Solution into aliquots according your need.</p>

* Elution Plates and Tip Plates are the same. Use one provided Elution Plate as a Tip Plate.

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

	1 x 96 extractions	5 x 96 extractions
Catalogue No.	7432300150	7432300250
Lysis Buffer G	50 ml	220 ml
Binding Buffer A	9 ml (final volume 30 ml)	36 ml (final volume 120 ml)
Proteinase K (working solution)	3 x 1 ml	13 ml
MAP Solution A	2 x 1.2 ml	10.5 ml*
Wash Buffer	2 x 45 ml final volume 2 x 150 ml	6 x 60 ml final volume 6 x 200 ml
Elution Buffer	30 ml	120 ml
1.5 ml Receiver Tubes	2 x 50	10 x 50
Sealing Foils	2	10
Manual	1	1
Initial steps	<p>Add 21 ml of 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.</p> <p>Dilute Proteinase K by addition of 1 ml of ddH₂O, mix thoroughly until completely dissolving</p> <p>Add 105 ml of 96-100% ethanol to the bottle Wash Buffer, mix thoroughly and keep the bottle always firmly closed!</p>	<p>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.</p> <p>Dilute Proteinase K by addition of 13 ml of ddH₂O, mix thoroughly until completely dissolving</p> <p>Add 140 ml of 96-100% ethanol to the bottle Wash Buffer, mix thoroughly and keep the bottle always firmly closed!</p> <p>*Divide the MAP Solution into aliquots according your need.</p>
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 Plates and Tip Plates are the same. Use one provided Elution Plate as a Tip Plate.

Symbols



Manufacturer



Lot number

Attention: Do not combine components of different kits, unless the lot numbers are identical!



Catalogue number



Expiry date



Consult operating instructions



Temperature limitation



Do not reuse



Humidity limitation

Storage

All buffers and kit contents of the **InviMag® Tissue DNA Mini Kit/ KF96**, except **dissolved Proteinase K** and **MAP Solution A** 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.

MAP Solution A: The magnetic beads should be stored at 2-8°C

Wash Buffer 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.

1 M DTT (not provided): Store the 1 M DTT solution at -20°C to prevent oxidative damage. The DTT solution is stable for 12 months at this condition.

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **InviMag® Tissue DNA Mini Kit/ KF96** for applications as described in this manual. Purchasers must determine the suitability of the product for its particular use. Should any product fail to perform in the applications described in the manual, STRATEC Molecular will check the lot and if a problem is approved, 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® Tissue DNA Mini Kit / KF96** is 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® Tissue 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® Tissue DNA Mini Kit/ KF96** is designed for semi-automated extraction and purification of total (genomic and mitochondrial) DNA from up to 96 tissue or tissue related samples using magnetic beads and the KF96 / KFflex96 instrument. The provided nucleic acid isolation protocols are suitable for semi-automated preparation of DNA from fresh or frozen tissue samples, biopsy samples, frozen sections, rodent tails, insects, , and eucaryotic cell pellets. For reproducible and high yields an appropriate sample storage is essential (see "Sampling and storage of the starting material", page 9). All utilities (reagents and plastic ware) necessary for preparation of total DNA are provided by the **InviMag® Tissue DNA Mini Kit/ KF96** in different package sizes.

The procedures of the **InviMag® Tissue DNA Mini Kit/ KF96** have been optimized for the isolation of total DNA from up to 20 mg tissue sample, app. 0.5 cm rodent tail or 1 x 10⁶ eucaryotic cells.

THE PRODUCT IS INTENDED FOR USE BY PROFESSIONALS 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 DNA 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 diagnostic results, adequate controls for downstream applications should be used.

The kit is in compliance with EU Directive 98/79/EC on in vitro medical devices. But it is not for in vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

Product use limitation

The kit is neither validated for the isolation of DNA from stool samples, whole blood, bacteria, fungi or viruses, nor for isolation and purification of RNA

The included chemicals are only useable once.

If the flow trace or starting material is changed, no issue in operability will be given.

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 under following conditions:

- If used in the US, based on the condition that the complete diagnostic system of the laboratory has been validated pursuant to CLIA' 88 regulations.
- For other countries based on the condition that the laboratory has been validated pursuant to equivalents according to the respective legal basis.

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 their detection.

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

The kit and its contents are unfit for consumption.

Safety information

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

Avoid direct 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.molecular.stratec.com for each STRATEC Molecular kit 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® Tissue 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, 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® Tissue DNA Mini Kit/ KF96** to which they apply, are listed below as follows:

Proteinase K



H315-319-334-335 P280-P305-P351-P338

Lysis Buffer G



Warning

H319-H412-P280-P305+P351+P338

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.H412: Harmful to aquatic life with long lasting effects.

H412: Harmful to aquatic life with long lasting effects.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 and easy to do. Continue rinsing.

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® Tissue DNA Mini Kit/ KF96

The **InviMag® Tissue DNA Mini Kit/ KF96** procedure is the ideal tool for an efficient DNA extraction and purification from fresh or frozen tissue samples, biopsy samples, frozen sections, rodent tails, insects, , and eucaryotic cell pellets in a convenient 96 well format using magnetic beads and the KF96 / KFflex96 workstation.

Starting material	Yield	Time for preparation	Ratio
up to 20 mg tissue samples, 0.5 cm rodent tail; up to 1x10 ⁶ mammalian cells	10 - 25 µg, depends on the starting material	app. 35 min (without lysis)	A ₂₆₀ :A ₂₈₀ 1.8 - 2.0

The DNA isolation process is based on the interaction of nucleic acids with coated magnetic particles at adapted buffer conditions. The KingFisher™ instrument performs all steps of the DNA purification procedure automatically without any user intervention, except the external lysis step. Due to the different possible starting materials, which require different starting protocols, an automated lysis step is not possible. However, the procedure requires only minimal interaction by the user. Sample cross-contamination and reagent cross-over is effectively eliminated by this automated purification process.

The KingFisher™ instrument uses magnetic rods to transport the DNA-binding magnetic particles through the various purification phases: binding, washing and elution. The buffer volumes and other liquids required for DNA isolation is reduced to a minimum.

After a sample specific cell lysis, using **Lysis Buffer G** and **Proteinase K**, optimal binding conditions are adjusted upon addition of **Binding Buffer A**. The DNA bound to the simultaneously added magnetic particles is then separated from solution by the magnetic rods, controlled by the KingFisher™ instrument. Subsequent to three washing steps of the particle bound nucleic acids, the DNA is eluted in **Elution Buffer**.

Due to the high purity, the eluted total (genomic and mitochondrial) DNA is ready-to-use for a broad panel of downstream applications like:

- PCR*
- Genotyping
- Restriction digestion
- SNP Analysis

The **InviMag® Tissue DNA Mini Kit/ KF96** is supplied with a comprehensive manual describing four protocols (page 14-15) for DNA purification from different sources.

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

For the isolation of DNA from a single tissue sample STRATEC Molecular offers the **Invisorb® Spin Tissue Mini Kit** or for 8-96 samples the **Invisorb® DNA Tissue Mini HTS 96 Kit/C** for use in a centrifuge (see "Ordering Information", page 24).

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

Sampling and sample storage of the starting material

For reproducible and high yields, appropriate sample storage is essential. Yields may be varying from sample to sample depending on factors such as sample age, kind of sample and storage conditions.

Tissue samples can be stored at room temperature (RT) for 2-3 hours. For short time 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 degrade the DNA.

Biopsy material / frozen section:

Best results are obtained with fresh material or material that has been immediately frozen and stored at -20°C or -80°C. Repeated freezing and thawing cycles of stored samples should be avoided because this can lead to a degraded DNA. Use of poor quality starting material influences the yield of purified DNA. The amount of purified DNA in the **InviMag® Tissue DNA Mini Kit/ KFmL** procedure using 5-20 mg tissue sample, depends on kind of starting material. The thawing process can be performed directly in **Lysis Buffer G**.

Rodent tail:

Best results are obtained with fresh material or material that has been immediately frozen and stored at -20°C or -80°C. Repeated freezing and thawing cycles of stored samples should be avoided because this can lead to a reduced DNA size. A prolonged lysis time may lead to degradation of DNA too. Crushing the rodent tails reduces the overall lysis time. An overnight lysis will probably shear the DNA, but is possible.

Insects:

Best results are obtained with fresh material or material that has been immediately frozen and stored at -20°C or -80°C. Insects must be homogenized before lysis (for example by grinding with mortar and pestle using liquid nitrogen) due to their stable chitin coat. The sample can be stored for a short time at 2-8°C in **Lysis Buffer G**.

Cells grown in suspension:

Spin up to 1×10^6 cells for 5 min at 300 x g (1.500 rpm). Discard the supernatant and remove all media completely. Do not disturb the cell pellet. At this point the cells may be frozen (at -80°C / -20°C) for future or immediately use.

Cells grown in a monolayer:

Aspirate the media completely from the cells and continue immediately with the lysis step. Alternatively the cells can be detached by trypsination (e.g. cultivation in larger culture vessels, like dishes > Ø 35 mm, flasks > 12.5 cm²). Transfer the cells to a 50 ml reaction tube, pellet by centrifugation at 300 x g (1.500 rpm) for 5 min and aspirate supernatant completely.

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

The **InviMag® Tissue DNA Mini Kit/ KF96** procedure comprises the following steps:

- Lysis of sample material and protein digestion
- Binding the genomic DNA to the magnetic beads
- Washing of the DNA and elimination of ethanol
- Elution of DNA

After lysis the DNA binds to the magnetic beads. Contaminations and enzyme inhibitors are efficiently removed during the following three wash steps and highly purified DNA is eluted in Elution Buffer.

This manual contains 4 protocols (pages 14-15).

Lysis

Samples are lysed at denaturing non-chaotropic conditions using elevated temperatures in the presence of **Lysis Buffer G** and **Proteinase K**. In case of a large sample number, the preparation of a master mixture with a volume 5% greater than that required for the processing of all samples is recommended. Mix the master mix carefully prior to use!

Binding of the DNA

After adding **Binding Buffer A** and **MAP Solution A** to the lysate in a 2.0 ml Deep Well Plate, the DNA is bound to the surface of the magnetic beads.

Removing residual contaminants

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

Elution

The DNA is eluted from the beads using 150 µl **Elution Buffer**. The eluted DNA is ready-to-use in different subsequent downstream applications such as PCR amplification, genotyping, restriction digestion and SNP analysis etc.

Yield and quality of genomic DNA

The amount of purified DNA in the **InviMag® Tissue DNA Mini Kit/ KF96** procedure from tissue or tissue related samples depends on the sample source, transport conditions, storage and age of the sample.

Yield and quality of isolated genomic DNA is suitable for any molecular-diagnostic detection system. The diagnostic tests should be performed accordingly to manufacturers' specifications.

Scheme of the InviMag® Tissue DNA Mini Kit/ KF96

Please read protocols prior the start of the preparation carefully

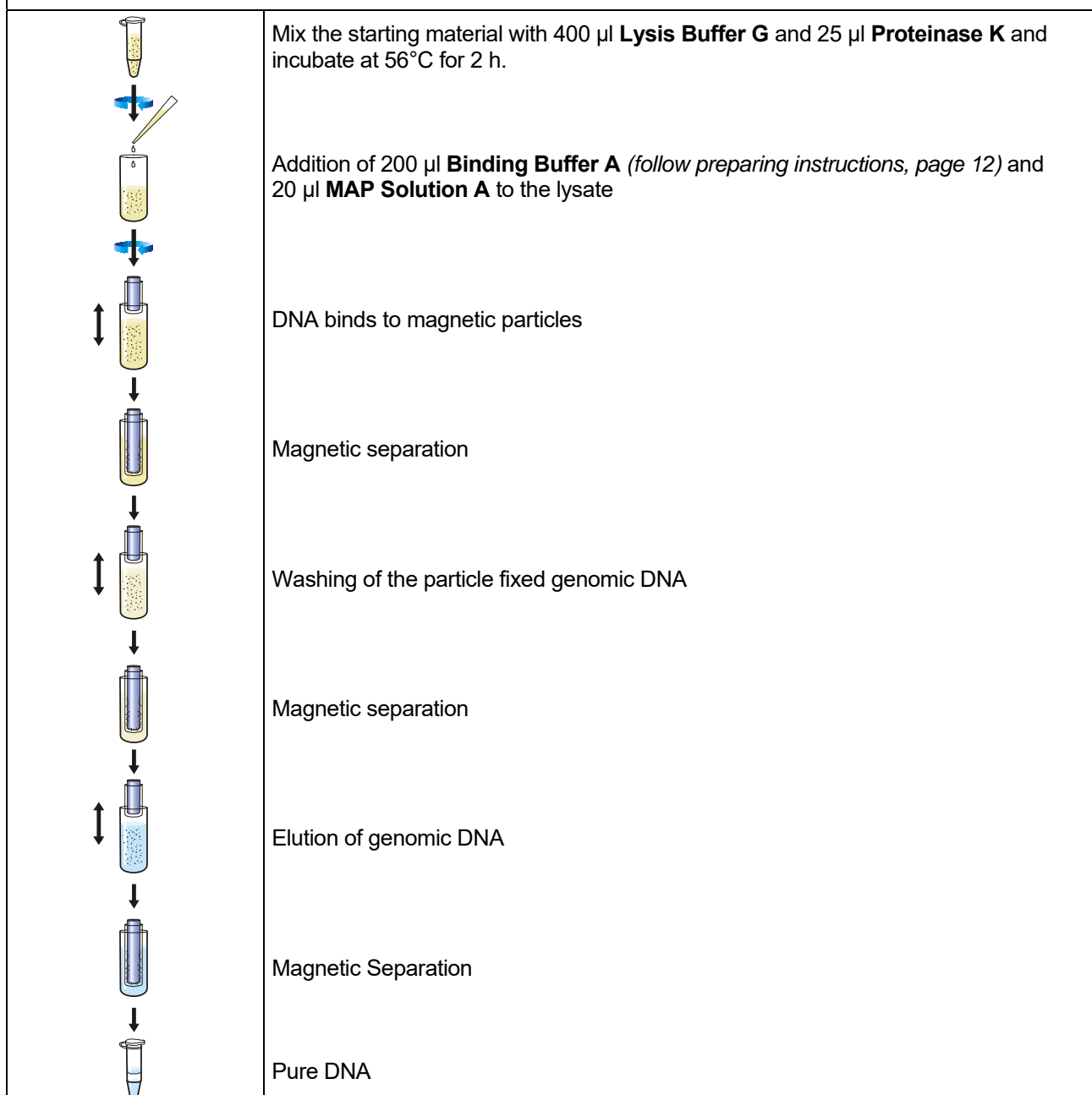
Transfer the sample into a 1.5 ml reaction tube (not provided) or into a cavity of a 2.0 ml Deep Well Plate (not provided). Add 400 µl Lysis Buffer G, add 25 µl Proteinase K to the sample and mix by vortexing.

Incubate for 2 h at 56°C or overnight at 52°C while continuously shaking. Spin down unlysed material.

Transfer each sample into a well of the 2.0 ml Deep Well Plate (refers to “Binding Plate”)

Prefill all plates of the KingFisher™ 96 with required buffers and the appropriate volumes.

Tip Plate:	Place in the KF 96 Tip Comb for DW magnets on a KF Elution Plate*
Binding Plate:	Add 200 µl Binding Buffer A and 20 µl MAP Solution A to the lysate
Washing Plate_1:	Add 800 µl Wash Buffer into a 2 ml Deep Well Plate
Washing Plate_2:	Add 800 µl Wash Buffer into a 2 ml Deep Well Plate
Washing Plate_3:	Add 800 µl Wash Buffer into a 2 ml Deep Well Plate
Elution Plate:	Add 150 µl Elution Buffer into the KF Elution Plate (same size as Tip Plate)



* Elution Plates and Tip Plates are the same. Use one provided Elution Plate as a Tip Plate.

Important notes

Important points before starting a protocol

After receiving the kit inspect the product and its components as well as the package for any visible damages and correct quantities. If there are any unconformities immediately notify STRATEC Molecular. 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 because their use may lead to poor kit performance.

- Always change pipet tips between liquid transfers. To avoid cross-contamination, we recommend the use of aerosol-barrier pipet tips.
- All centrifugation steps are carried out at room temperature.
- 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.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow until the samples are lysed.
- The kit should only be used by trained personnel.

Preparing reagents and buffers

Before starting a run, bring all reagents to room temperature. Where necessary, gently mix and re-dissolve any precipitates by warming up to 30°C. Swirl gently to avoid foaming.

Lysis Buffer G and **Elution Buffer** are ready to use.

1 x 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 ml of ddH₂O, mix thoroughly until completely dissolving
Add 105 ml of 96-100% ethanol to the bottle **Wash Buffer**, mix thoroughly and keep the bottle always firmly closed!

5 x 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 13 ml of ddH₂O, mix thoroughly until completely dissolving
Add 140 ml of 96-100% ethanol to the bottle **Wash Buffer**, mix thoroughly and keep the bottle always firmly closed!

Reagents and equipment to be supplied by user

- Measuring cylinder (250 ml)
- Pipette and pipette tips
- Disposable gloves
- Reaction tubes (1.5 or 2.0 ml)
- Vortexer
- ddH₂O
- 96-100% ethanol
- Isopropanol

The **InviMag® Tissue 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 No. A3928

Sigma Aldrich
2-Propanol
Order No. 59304-1L-F

Important indications for the InviMag® Tissue DNA Mini Kit/ KF96

1. From liver samples only use up to 20 mg of tissue. Excessive amount of tissue will result in inefficient lysis. 20 mg tissue will provide an average yield of 4-35 µg genomic DNA.
2. Incomplete removal of the cell culture media will dilute the lysate and affect lysis.
3. Optimal disruption of the tissue sample is important for obtaining maximum yields and purity of the genomic DNA. The most common method of cell disruption is by grinding with a mortar and pestle using liquid nitrogen. With this method, it is possible to reduce the incubation time with **Proteinase K**. Alternatively a homogenizer for disruption of freshly extracted tissue samples can be used. Homogenization of the tissue sample in **Lysis Buffer G** is recommended.
4. The simultaneous isolation of RNA by the **InviMag® Tissue DNA Mini Kit/ KF96** cannot be excluded in all experimental setups. If removal of RNA contaminations is required, add 40 µl RNase A (stock solution 10 mg/ml) before the addition of **Binding Buffer A**. Vortex shortly and incubate the sample at room temperature for 5 min. Then continue with the protocol description.
5. The elution can be done by using reduced volumes of **Elution Buffer** (minimal 100 µl). This may result in a higher DNA-concentration, but will reduce the total yield. Please keep in mind that a change of volume has to be changed in the provided assay file too by using the Thermo BindIt software.

Protocol 1: Isolation of genomic DNA from up to 20 mg tissue

Please read the instructions carefully and conduct the prepared procedure!

Attention: Please be aware, that the **Binding Buffer A and Wash Buffer** have to be prepared – see instructions, page 12

I. Sample Lysis

A. Sample lysis in a 1.5 ml reaction tube (not provided)

1. Cut the tissue sample (app. 0.5 cm of the mouse tail or up to 20 mg tissue), the biopsy sample, frozen section or insects into small pieces or homogenize the starting material.
2. Transfer the tissue sample into a 1.5 ml reaction tube.
3. Add 400 µl of **Lysis Buffer G** and 25 µl **Proteinase K**.

Important: Vortex the tube for 5 s! An incomplete mixing will reduce quality and yield of the isolated DNA.

4. Incubate the sample at 56°C for app. 2 h (or overnight at 52°C) while continuously shaking (e.g. by using a thermomixer).
5. During lysis, prefill all plates with required buffers and appropriate volumes (see “Starting a Run”, page 17).
6. After lysis, centrifuge the sample for 2 min at max. speed and transfer the cleared supernatant into the cavity of the Binding Plate prefilled with 200 µl **Binding Buffer A** and 20 µl **MAP Solution A** (see “Starting a Run”, page 17).

B. Sample lysis in a Deep Well Plate (not provided)

1. Cut the tissue (app. 0.5 cm of the mouse tail or up to 20 mg tissue) into small pieces.
2. Transfer the tissue sample into the cavities of the Deep Well Plate (refers to “Binding Plate”) and add 400 µl **Lysis Buffer G** and 25 µl **Proteinase K**.
3. Seal the Deep Well Plate with a Sealing Foil.

Important Note: Vortex the Deep Well Plate 10 times. An incomplete mixing will reduce quality and yield of the isolated DNA.

4. Incubate the Deep Well Plate at 56°C for 2 h or overnight at 52°C (use e.g. hybridization oven or incubator) until lysis is complete.

Important Note: Vortex the Deep Well Plate each 20 minutes for 20 sec. Vortexing is essentially for an efficient lysis step. No vortexing will lead to a dramatic reduction of the final yield.

5. During lysis, prefill all plates with the required buffers and appropriate volumes (see “Starting a Run”, page 17).
6. After lysis, centrifuge the Deep Well Plate with the lysed samples for 10 min at 4.000 rpm to spin down the unlysed material.
7. Transfer the cleared supernatant of the lysate carefully into the cavity of the Binding Plate prefilled with 200 µl **Binding Buffer A** and 20 µl **MAP Solution A** (see “Starting a Run”, page 17).

Protocol 2: Isolation and purification of DNA from eucaryotic cells

Please read the instructions carefully and conduct the prepared procedure!

Attention: Please be aware, that the **Binding Buffer A and Wash Buffer** have to be prepared – see instructions, page 12

Harvesting cells

Cells grown in suspension: Spin up to 1×10^6 cells for 5 min at $300 \times g$. (1500 rpm)
Discard the supernatant and carefully remove all media.

Cells grown in a monolayer: In *large culture vessels* (dishes $> \varnothing 35$ mm, flasks > 12.5 cm²) detach cells by trypsination. Transfer the cells to a centrifuge tube and sediment the cell by centrifugation at 1500 rpm for 5 min. Remove the supernatant completely.

In *small culture vessels* (96-, 24-, 12-, 6-well plates, $\varnothing 35$ mm dishes, 12.5 cm² flasks) discard the media completely and continue with the lysis immediately.

I. Sample Lysis

A. Sample lysis in a 1.5 ml reaction tube (*not provided*)

1. Transfer up to 1×10^6 cells into a 1.5 ml reaction tube.
2. Spin down for 5 min at $300 \times g$ (1500 rpm).
3. Discard the supernatant and remove all media completely. Do not disturb the cell pellet.
4. Add 400 μ l of **Lysis Buffer G** and 25 μ l **Proteinase K**.

Important: Vortex the tube for 5 s! An incomplete mixing will reduce the quality and yield of the isolated DNA.

5. Incubate the sample at 56°C for 2 h while continuously shaking (e.g. by using a thermomixer).
6. During lysis, prefill all plates with the required buffers and appropriate volumes (see “Starting a Run”, page 17).
7. After lysis, centrifuge the sample for 2 min at max. speed and transfer the cleared supernatant carefully into the cavity of the Binding Plate pre-filled with 200 μ l **Binding Buffer A** and 20 μ l **MAP Solution A** (see “Starting a Run”, page 17).

B. Sample lysis in a Deep Well Plate

1. Transfer up to 1×10^6 cells into the cavity of a Deep Well Plate.
2. Spin down for 5 min at $300 \times g$ (1500 rpm).
3. Discard the supernatant and remove all media completely. Do not disturb the cell pellet.
4. Add 400 μ l **Lysis Buffer G** and 25 μ l **Proteinase K**.
5. Seal the Deep Well Plate with a Sealing Foil.

Important Note: Vortex the Deep Well Plate 10 times. An incomplete mixing will reduce quality and yield of the isolated DNA.

6. Incubate the Deep Well Plate at 56°C for 2 h (e.g. use a hybridization oven or incubator) until lysis is complete.

Important Note: *Vortex the Deep Well Plate each 20 minutes for 20 sec. Vortexing is essentially for an efficient lysis step. No vortexing will lead to a significant reduction of the final yield.*

7. During lysis, prefill all plates with the required buffers and appropriate volumes (see “Starting a Run”, page 17).
8. After lysis, centrifuge the Deep Well Plate with the lysed samples for 10 min at 1700 x g (4000 rpm) to spin down unlysed material.
9. Carefully transfer the cleared supernatant from the lysate into the cavity of the Binding Plate prefilled with 200 µl **Binding Buffer A** and 20 µl **MAP Solution A** (see “Starting a Run”, page 18).

Starting a run on a KF96 / KFflex96 instrument

Preliminary Steps to process the sample onto the KingFisher™ System

Attention: Please be aware, that the **Binding Buffer A and Wash Buffer** have to be prepared – see instructions, page 12

Important: Before working with a KingFisher™ instrument please carefully read the manufacturer's manual!

1. Switch on the KF96 / KFflex96 instrument

Note: Use one provided Elution Plate as Tip Plate. These are identical.

2. Prefill the Deep Well Plates with the corresponding buffers during the lysis.

Note: Please avoid evaporation of the prefilled buffer components by sealing the Deep Well Plates with a sealing foil or with parafilm!

Important: Mix the bottle with the **MAP Solution A** by vigorously vortexing!

Tip Plate: Place the Tip Plate with a KF 96 Tip Comb for DW magnets on an Elution Plate

Binding Plate: Transfer the cleared lysate carefully into the cavity of a 2 ml DWP prefilled with 200 µl **Binding Buffer A** and 20 µl **MAP Solution A**.

Washing Plate_1: Pipet 800 µl **Wash Buffer** into a 2 ml Deep Well Plate

Washing Plate_2: Pipet 800 µl **Wash Buffer** into a 2 ml Deep Well Plate

Washing Plate_3: Pipet 800 µl **Wash Buffer** into a 2 ml Deep Well Plate

Elution Plate Pipet 150 µl **Elution Buffer** into the KF Elution Plate

3. Choose the program “**InviMag Tissue DNA KF96**” or “**InviMag Tissue DNA KFflex96**” depending on the used instrument press the “START” button
4. Insert the prefilled Plates onto the right position of instrument surface by following the specification shown on the display and confirm every loading step by pressing the “START” button.
5. When all prefilled plates are loaded into the machine, press the “START” button finally to begin the run.

III. The following extraction steps run automatically on the KingFisher™ System

Binding of the DNA

Automatically sample mixing for 5 min. MAP separation. Moving of the MAP into Washing Plate 1.

First Washing

Automatically sample mixing for 90 s. MAP separation. Moving of the MAP into the Washing Plate_2.

Second Washing

Automatically sample mixing for 90 s. MAP separation. Moving of the MAP into Washing Plate 3.

Third Washing and Drying

Automatically sample mixing for 90 s. MAP separation. Air-drying of the MAP on the Washing Plate 3 for 5 min. Moving of the MAP into the Elution Plate.

Elution of the DNA

Incubation of the MAP into the Elution Plate for 10 min by mixing at 70°C. MAP separation.

The MAP will then be removed into the Washing Plate 3 (disposal).

The extracted DNA can now be transferred into new 1.5 ml **Receiver Tubes**.

Important Notes: *After finishing the extraction protocol, the Elution Plate contains the extracted genomic DNA. Store the DNA under adequate conditions. We recommend to transfer the extracted DNA into 1.5 ml Receiver Tubes for further storage and freeze the DNA at -20°C.*

If the extracted DNA contains carryover of magnetic particle, transfer the DNA to 1.5 ml reaction tube, centrifuge at maximum speed (13000 rpm) for 1 min and transfer the DNA-containing supernatant into a receiver tube.

For self-programming of the KF96 and 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	
Lysed sample	425	-	Sample	
Binding Buffer A	200	-	Reagent	
Map Solution A	20	-	Reagent	
Washing Plate 1		Microtiter DW 96 plate		
Name	Well volume [µl]	Total reagent volume [µl]	Type	
Wash Buffer	800	-	Reagent	
Washing Plate 2		Microtiter DW 96 plate		
Name	Well volume [µl]	Total reagent volume [µl]	Type	
Wash Buffer	800	-	Reagent	
Washing Plate 3		Microtiter DW 96 plate		
Name	Well volume [µl]	Total reagent volume [µl]	Type	
Wash Buffer	800	-	Reagent	
Elution Plate		KingFisher 96 KF plate		
Name	Well volume [µl]	Total reagent volume [µl]	Type	
Elution Buffer	150	-	Reagent	

Dispensed reagents

The protocol does not contain dispensed reagents

Steps data

	Tip	96 DW tip comb	
	Pick-Up	Tip Plate	
	Binding	Binding Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:10, Fast
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	Wash1	Washing Plate 1	
	Beginning of step	Precollect	No
		Release time, speed	00:00:30, 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
	Wash2	Washing Plate 2	
	Beginning of step	Precollect	No
		Release time, speed	00:00:30, Medium
	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
	Wash3	Washing Plate 3	
	Beginning of step	Precollect	No
		Release time, speed	00:00:30, Medium
	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
	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]	70
		Preheat	Yes
	End of step	Postmix	No
		Collect count	4
		Collect time [s]	5
	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	<p>insufficient lysis</p> <p>incomplete elution</p> <p>low amount of MAP Solution A</p>	<p>increase lyses time. reduce amount of starting material</p> <p>use a higher volume of Elution Buffer, be sure you pipet the Elution Buffer with the correct amount to the correct position</p> <p>mix MAP Solution A thoroughly before pipetting to the Binding Plate</p>
low concentration of extracted DNA	<p>too much Elution Buffer</p> <p>incorrect storage of starting material</p>	<p>elute the DNA with lower volume of Elution Buffer</p> <p>ensure that the storage of starting material was correctly Avoid thawing of the material</p>
degraded or sheared DNA	<p>incorrect storage of starting material</p> <p>old material</p>	<p>ensure that the storage of starting material was correctly Avoid thawing of the material</p> <p>ensure that the starting material is fresh or stored under appropriate condition (for long time storage at -20°C)!</p> <p>avoid thawing and freezing of the material</p> <p>old material often contains degraded DNA</p>
DNA does not perform well in downstream-applications (e.g. real-time PCR or PCR)	<p>ethanol carryover during elution</p> <p>salt carry-over during elution</p>	<p>increase drying time for removing of ethanol</p> <p>check the Wash Buffers for salt precipitates. If there are any precipitates visible, solve them by carefully warming up to 30°C</p> <p>ensure that the Wash Buffers are at room temperature</p>
low $A_{260}:A_{280}$ ratio from UV measurement, eluted DNA is brown colored	<p>small part of the magnetic particles are left in the elution</p>	<p>centrifuge at full speed for 1 min and transfer supernatant to a new tube</p>

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 require 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 necessary to ensure it will function well in various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, long-template PCR, and construction of cosmid libraries.

Handling fresh and stored material before the extraction of DNA

For the isolation of genomic DNA from cells or tissues, use either fresh samples or samples that have been quickly frozen in liquid nitrogen and stored at -70°C . This procedure minimizes degradation of crude DNA by limiting the activity of endogenous nucleases.

Storage of DNA

Store genomic DNA at $2-8^{\circ}\text{C}$. Storing genomic DNA at -20°C can cause shearing of DNA, particularly if the DNA is exposed to repeated freeze-thaw cycles. Plasmid DNA and other small circular DNAs can be stored at $2-8^{\circ}\text{C}$ or at -20°C .

Drying, dissolving and pipetting DNA

Avoid overdrying genomic DNA after ethanol precipitation. It is better to let it air dry than to use a vacuum, although vacuum drying can be used with caution. Plasmid DNA and other small circular DNAs can be vacuum-dried.

To help dissolve the DNA, carefully invert the tubes several times after adding buffer and tap the tube gently on the side. Alternatively let the DNA stand in buffer overnight at $2-8^{\circ}\text{C}$. Minimize vortexing of genomic DNA since this can cause shearing.

Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings causes 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. Regular pipette tips pose no problem for plasmid DNA and other small.

Ordering Information

Product	Package size	Catalogue No.
InviMag® Tissue DNA Mini Kit/ KF96	1 x 96 preparations	7432300100
InviMag® Tissue DNA Mini Kit/ KF96	5 x 96 preparations	7432300200

Single components for InviMag® Tissue DNA Mini Kit/ KF96

Lysis Buffer G	60 ml	7432301300
Binding Buffer A	30 ml	7432302700
MAP Solution A	1 ml	7432305100
Wash Buffer (add 140 ml ethanol)	60 ml	7432303200
Elution Buffer	30 ml	7432304000

KingFisher™ 96 and consumables

KingFisher 96, Magnetic Particle Processor, 100-240V, 50/60Hz (including one magnetic head)		5400500
KingFisher 96 Head for Deep Well plate		24073430
KingFisher 96 tip comb for PCR magnets, 8 x 10 pcs / box		97002514
KingFisher 96 tip comb for KF magnets, 10 x 10 pcs / box		97002524
KingFisher 96 tip comb for DW magnets 10 x 10 pcs / box		97002534
KingFisher 96 KF plate (200ul) 48 plates / box		97002540
Microtiter deep well 96 plate, 50 plates / box		95040450

Related Products	Package size	Catalogue No.
Invisorb® Spin Tissue Mini Kit	50 preparations	1032100200
Invisorb® Spin Tissue Mini Kit	250 preparations	1032100300
Invisorb® DNA Tissue HTS 96 Kit/ C	4 x 96 preparations	7032300300
Invisorb® DNA Tissue HTS 96 Kit/ C	24 x 96 preparations	7032300400

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

Fa. Sigma
2-Propanol
Order No. 59304-1L-F



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