Instruction InviMag® Blood DNA Mini Kit/ KFmL

The **InviMag® Blood DNA Mini Kit/ KFmL** 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, an uniform size distribution, and a good suspension stability. They are highly suitable for high-throughput processing.

The **InviMag® Blood DNA Mini Kit/ KFmL** for isolation and purification of total (genomic and mitochondrial) DNA from whole blood samples, buffy coat, non-mammalian blood, cerebrospinal fluid (CSF), bone marrow, and swabs in a single well format for up to 15 samples per run has been designed for an optimal use on the KingFisher® mL workstation from Thermo Scientific. The interplay of the DNA extraction and purification chemistry provided by the **InviMag® Blood DNA Mini Kit/ KFmL** with the KingFisher machine was intensely tested and validated.

The kit is neither suitable for isolation of DNA from stool samples, tissue samples, bacteria, fungi, plants or viruses nor for purification of RNA.

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

Content

Kit contents of InviMag® Blood DNA Mini Kit/ KFmL

Store the SNAP Solution at 4°C!
Store lyophilized Proteinase K at 2-8°C!
Store dissolved Proteinase K at -20°C!
Store all other kit components at room temperature (RT)!

	15 extractions	**75 extractions**	**150 extractions**	**300extractions**
Catalogue No.	2431110100	2431110200	2431110300	2431110400
Lysis Buffer A	5 ml	20 ml	40 ml	70 ml
Proteinase K (working solution)	for 0.5 ml working solution	for 2 ml working solution	for 2 x 2 ml working solution	for 7 ml working solution
SNAP Solution	0.5 ml	2 x 1 ml	3 x 1.2 ml	6 x 1.2 ml
Binding Buffer B6	8 ml	40 ml	70 ml	140 ml
Wash Buffer I	7.5 ml (final volume 15 ml)	2 x 30 ml (final volume 2 x 60 ml)	80 ml (final volume 160 ml)	2 x 80 ml (final volume 2 x 160 ml)
Wash Buffer II	18 ml (final volume 60 ml)	45 ml (final volume 150 ml)	2 x 45 ml (final volume 2 x 150 ml)	3 x 60 ml (final volume 3 x 200 ml)
Elution Buffer D	2 x 2 ml	20 ml	40 ml	70 ml
KingFisher mL Tip Combs	3	15	30	60
KingFisher mL Tube Strips	15	5 x 15	150	300
1.5 ml Receiver Tubes	15	5 x 15	3 x 50	6 x 50
Manual	1	1	1	1
Initial steps	Dilute **Proteinase K** by addition of 0.5 ml ddH_2O, mix thoroughly and store like described below! Add 7.5 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and always keep the bottle firmly closed! Add 42 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and always keep the bottle firmly closed!	Dilute **Proteinase K** by addition of 2 ml ddH_2O, mix thoroughly and store like described below! Add 30 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and always keep the bottle firmly closed! Add 105 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and always keep the bottle firmly closed!	Dilute **Proteinase K** by addition of each 2 ml ddH_2O, mix thoroughly and store like described below! Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and always keep the bottle firmly closed! Add 105 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and always keep the bottle firmly closed!	Dilute **Proteinase K** by addition of 7 ml ddH_2O, mix thoroughly and store like described below! Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer I**, mix thoroughly and always keep the bottle firmly closed! Add 140 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and always keep the bottle firmly closed!

Kit contents of InviMag® Blood DNA Mini Kit/ KFmL/ wp

Store the SNAP Solution at 4°C!
Store lyophilized Proteinase K at 2-8°C!
Store dissolved Proteinase K at -20°C!
Store all other kit components at room temperature (RT)!

	15 extractions	**75 extractions**	**150 extractions**	**300extractions**
Catalogue No.	2431110150	2431110250	2431110350	2431110450
Lysis Buffer A	5 ml	20 ml	40 ml	70 ml
Proteinase K (working solution)	for 0.5 ml working solution	for 2 ml working solution	for 2 x 2 ml working solution	for 7 ml working solution
SNAP Solution	0.5 ml	2 x 1 ml	3 x 1.2 ml	6 x 1.2 ml
Binding Buffer B6	8 ml	40 ml	70 ml	140 ml
Wash Buffer I	7.5 ml (final volume 15 ml)	2 x 30 ml (final volume 2 x 60 ml)	80 ml (final volume 160 ml)	2 x 80 ml (final volume 2 x 160 ml)
Wash Buffer II	18 ml (final volume 60 ml)	45 ml (final volume 150 ml)	2 x 45 ml (final volume 2 x 150 ml)	3 x 60 ml (final volume 3 x 200 ml)
Elution Buffer D	2 x 2 ml	20 ml	40 ml	70 ml
1.5 ml Receiver Tubes	15	5 x 15	3 x 50	6 x 50
Manual	1	1	1	1
Initial steps	Dilute **Proteinase K** by addition of 0.5 ml ddH_2O, mix thoroughly and store like described below! Add 7.5 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and always keep the bottle firmly closed! Add 42 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and always keep the bottle firmly closed!	Dilute **Proteinase K** by addition of 2 ml ddH_2O, mix thoroughly and store like described below! Add 30 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and always keep the bottle firmly closed! Add 105 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and always keep the bottle firmly closed!	Dilute **Proteinase K** by addition of each 2 ml ddH_2O, mix thoroughly and store like described below! Add 80 ml of 96-100% ethanol to the bottle **Wash Buffer I**, mix thoroughly and always keep the bottle firmly closed! Add 105 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and always keep the bottle firmly closed!	Dilute **Proteinase K** by addition of 7 ml ddH_2O, mix thoroughly and store like described below! Add 80 ml of 96-100% ethanol to each bottle **Wash Buffer I**, mix thoroughly and always keep the bottle firmly closed! Add 140 ml of 96-100% ethanol to each bottle **Wash Buffer II**, mix thoroughly and always keep the bottle firmly closed!
Plastic to be supplied by user (see order information)				
KingFisher mL Tip Combs	3	15	30	60
KingFisher mL Tube Strips	15	5 x 15	150	300

Symbols

 manufacturer

 lot number

 catalogue number

 date of manufacture

 expiry date

 consult operating instructions

 temperature limitation

 do not reuse

Storage

All buffers and kit contents of the InviMag® Blood DNA Mini Kit/ KFmL, except **Proteinase K** and **SNAP Solution** should be stored at room temperature* and are stable for at least 12 months under these conditions.

Proteinase K: Lyophilized Proteinase K should be stored at 2-8°C. Dissolved Proteinase K must be stored at -20℃. Dividing the Proteinase K into aliquots and storage at -20℃ is recommended.

SNAP Solution: SNAP Solution should be stored at 4℃.

Wash Buffers: Wash Buffers charged with ethanol should be stored at room temperature* and should be appropriate sealed. If there are any precipitates visible within the provided solutions solve them by carefully warming up to room temperature (up to 30℃).

*Room temperature (RT) is defined as range from 15-30℃.

Quality control

STRATEC Molecular guarantees the correct function of the InviMag® Blood DNA Mini Kit/ KFmL for applications as described in the manual. In accordance with STRATEC Molecular's certified QM-System each component of the InviMag® Blood DNA Mini Kit/ KFmL was tested against predetermined specifications to ensure consistent product quality.
All products sold by STRATEC Molecular are subjected to extensive quality control procedures according to ISO 9001-2000 and are warranted to perform as described when used correctly. Any problems should be reported immediately.
If you have any questions or problems regarding any aspects of InviMag® Blood DNA Mini Kit/ KFmL or other STRATEC Molecular products, please do not hesitate to contact us.

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® Blood DNA Mini Kit/ KFmL** has been designed for semi-automated extraction and purification of total (genomic and mitochondrial) DNA from up to 15 whole blood or blood related samples using magnetic beads and the KingFisher mL instrument. The nucleic acid isolation protocol is suitable for routinely walk-away automated preparation of DNA from fresh or frozen whole blood sample, buffy coat, non mammalian blood, cerebrospinal fluid (CSF), bone marrow, and swabs. For reproducible and high yields an appropriate sample storage is essential (see “Sampling and storage of the starting material”, page 9). Common blood collection tubes (not provided) and anticoagulants (EDTA, citrate, but ***not heparin***) can be used to assemble a set of blood samples. All utilities (reagents and plastic ware) necessary for preparation of total DNA are provided by the **InviMag® Blood DNA Mini Kit/ KFmL** in different package sizes.

The procedure of the **InviMag® Blood DNA Mini Kit/ KFmL** has been optimized for the isolation of total DNA from up to 200 µl starting material. For samples of a smaller volume than 200 µl please adjust to a total sample volume of 200 µl using distilled water or 1x PBS before starting a purification protocol.

Any diagnostic results generated, using the sample preparation procedure in conjunction with any downstream diagnostic assays, should be interpreted with regard to other clinical or laboratory finding.

The product is intented for use by professional users 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 modification of DNA followed by signal detection or amplification. Any diagnostic results generated 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.

Product use limitation

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

The included chemicals are for single use only.

When differing the starting material or the flow trace, no guarantee in operability is issued.

The user is responsible to validate the performance of the STRATEC Molecular kits for any particular use, since the performance characteristics of our kits have not been validated for any specific application. STRATEC Molecular kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA’ 88 regulations in the U.S. or equivalents in other countries.

All products sold by STRATEC Molecular are subjected to extensive quality control procedures (according to EN ISO 9001-2000 and EN ISO 13485-2003) and are warranted to perform as described when used correctly. Any problems should be reported immediately.

The chemicals and plastic parts are for laboratory use only, they have to be stored in the laboratory and has not to be used for other purposes than intended.

The kit contents are not suitable for consumption.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. Heed the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). They are available online in convenient and compact PDF format at **www.invitek.de** under each STRATEC Molecular kit and whose kit component.

If the 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® Blood DNA Mini Kit/ KFmL** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be exclude completely. Therefore, liquid waste have be considered infectious and be handled and discarded according to local safety regulation.

Below European Community risk and safety phrases for the components of the **InviMag® Blood DNA Mini Kit/ KFmL** to which they apply, are listed.

Wash Buffer I contains guanidine thiocyanate which is an irritant.

Lysis Buffer A

danger
H-319 P305-351-338

Binding Buffer B6

danger
H225-319-336 P210-233-305-351-338

Proteinase K

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

Wash Buffer I

warning
H302-312-332-412 EUH032 P273

H319:	Causes serious eye irritation.
H225:	Highly flammable liquid and vapor.
H336:	May cause drowsiness or dizziness.
H315:	Causes skin 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.
P305+P351+P338:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P210:	Keep away from heat/sparks/open flames/hot surfaces. — No smoking.
P233:	Keep container tightly closed.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.
P273:	Avoid release to the environment.

Emergency medical information in English and German can be obtained 24 hours a day from:
Poison Information Center Freiburg, Germany: Phone: +49 761 19240

Product characteristics of the InviMag® Blood DNA Mini Kit/ KFmL

The **InviMag® Blood DNA Mini Kit/ KFmL** procedure the ideal tool for an efficient DNA extraction and purification from fresh or frozen whole blood samples, non-mammalian blood, buffy coat, CST, bone marrow, and swabs in a single well format for up to 15 samples per run using magnetic beads and the KingFisher mL.

Starting material	Yield	Time	Ratio
1-200 µl fresh, frozen or old human or other mammalian whole blood; 1-200 µl cerebrospinal fluid; 1–30 µl buffy coat; 1–25 µl fresh, frozen or old non mammalian blood; 1–20 µl bone marrow; swabs; up to 200 µl rinsed liquid from swab	up to 10 µg (in average abou 6 µg) depends on amount o lymphocytes, sample source sample transport, sample storage, and age of the sample	30 min (without lysis)	A_{260} : A_{280} 1.7 – 2.0

The DNA isolation process is based on the interaction of nucleic acids with coated magnetic particles under adapted buffer conditions. After lysis, the KingFisher mL performs all steps of the DNA purification procedure automatically without any user intervention. The procedure requires minimal interaction by the user, thus allowing safe handling of potentially infectious samples. Sample cross-contaminations and reagent cross-over are effectively eliminated by this automated purification process.

The KingFisher® instruments use magnetic rods to transport the DNA-binding magnetic particles through the various purification phases: binding-washing-elution. The volume of buffers and other liquids necessary for DNA isolation is reduced to a minimum.
After an external sample specific cell lysis using Lysis Buffer A and Proteinase K, optimal binding conditions are adjusted upon addition of Binding Solution B6. The total DNA bound to the simultaneously added magnetic particles is separated from solution by the magnetic rods controlled by the KingFisher instrument. Subsequent to the three washing steps of the particle bound nucleic acids, the DNA is finally eluted in Elution Buffer D.

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

- PCR*
- Restriction Enzyme Digestion
- HLA Typing
- Southern Blot.

The **InviMag® Blood DNA Mini Kit/ KFmL** is supplied with a comprehensive manual describing four protocols (page 13-14) 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 genomic DNA using magnetic particles in a 96 well format, STRATEC Molecular offers the **InviMag® Blood DNA Mini Kit/ KF96/ KFflex96** for use on a KF96 / KFflex96 instrument. For the isolation of DNA from a single blood sample STRATEC Molecular offers the **Invisorb® Spin Blood Mini Kit** or for 8-96 samples the **Invisorb® DNA Blood Mini HTS 96 Kits** for use on a centrifuge, vacuum manifold or other robotic stations (see “Ordering Information”, page 22).

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

Sampling and sample storage of the starting material

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

Blood and Buffy Coat:

Best results are obtained using fresh blood samples. Mammalian blood samples (stabilized with EDTA or citrate but *not heparin*) can be stored at room temperature for up to 2-3 hours. For short-term storage (up to 24 h) samples should be stored at 4°C. For long-term storage, we recommend freezing samples at -20°C or -80°C. Multi ple thawing and freezing cycles before isolating the DNA should be avoided. If cryoprecipitates (formed during thawing of frozen samples) are visible avoid aspirating them. Various different primary tubes, blood collection system (e.g. Sarstedt, Greiner) and anticoagulants (*except heparin*) can be used to collect blood samples for the **InviMag® Blood DNA Mini Kit/ KFmL** procedure.

Buffy coat is a whole-blood fraction of enriched leukocyte cells. To prepare and extract of buffy coat the following procedure is recommended: The use of a whole blood sample (anticoagulants: EDTA, citrate, *not heparin*) with a sedimented cellular fraction from staying overnight at 4°C is recommended. The resulting brig ht mid-section overlaid by the clear plasma is the buffy coat containing concentrated leukocytes that can be easily distinguished from the erythrocytes in the bottom layer. An enrichment factor of 10 is expected from such a procedure. Due to the enriched leukocyte content be aware to avoid overloading the purification system.

CSF (Cerebrospinal fluid) and Bone marrow:

Best results are obtained with fresh material which can be stored for 2-3 h at 4°C or for long-term storage freeze the sample at -20°C. However, o ften the sample is dried. Dried samples have to be stored at 4°C in a dry surrounding.

Swabs

The protocol works with fresh prepared swabs as well as with dried swabs. Please note, that stored and dried swabs can lead to samples that show an apoptotic DNA ladder (visible on agarose gel as typical apoptotic DNA banding pattern).The protocol has not been validated for isolation of DNA from swabs which are stored in special storage buffers from other providers.

Principle and procedure

The **InviMag® Blood DNA Mini Kit/ KFmL** procedure comprises following steps:

- lysis of sample material and protein digestion
- binding the genomic DNA to the magnetic beads
- washing the beads and elimination of ethanol
- elution of genomic DNA

After lysis, the DNA binds to the magnetic beads whereas contaminations and inhibitors are efficiently removed during the following three wash steps. Highly purified DNA is eluted in Elution Buffer D.

This manual contains 4 protocols (page 13-14).

Lysis

Lysis is performed at elevated temperatures in the presence of **Lysis Buffer A** and **Proteinase K**. In case of large sample numbers we recommend the preparation of a master mix with a volume 5% greater than that required. Carefully mix the master mix carefully prior to use!

Binding of the genomic DNA

After adding **Binding Buffer B6** and **SNAP Solution** to the lysate the DNA is bound to the surface of the magnetic beads.

Removing residual contaminants

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

Elution

The DNA is eluted in 200 µl **Elution Buffer D**. The eluted DNA is ready-to-use in different subsequent downstream applications e.g. for PCR amplification, digestion with restriction enzymes, Southern hybridizations, HLA typing etc.

Yield and qualit of genomic DNA

The amount of purified DNA in the **InviMag® Blood DNA Mini Kit/ KFmL** procedure from whole blood depends on the leucocytes content, sample source, transport, storage, and sample age.

Typically, a 200 µl sample of whole blood cells (samples with elevated white blood cell (WBC counts), ranging from $3x10^6$ to $1x10^7$ cells/ml) from a healthy individual will yield 3–12 µg of DNA. The typical yield usually derived from the **InviMag® Blood DNA Mini Kit/ KFmL** is about 3-8 µg of DNA. If the whole blood sample is mixed with anticoagulant containing buffers the overall leukocyte concentration decreases and the yield of the DNA extraction procedure is reduced.

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

Important points before starting a protocol

After receiving the kit, check the kit components for damage. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to "Safety information" (page 7). Do not use damaged kit components because thy may lead to poor kit performance.

- always change pipet tips between liquid transfer, 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
- this kit should only be used by trained personnel

Preparing reagents and buffers

Before starting a run, equilibrate all reagents at room temperature. Where necessary, gently mix and redissolve any precipitates by an incubation at 30℃. Swirl gently to avoid foaming.

Lysis Buffer A, Binding Buffer B6 and **Elution Buffer D** are ready-to-use.

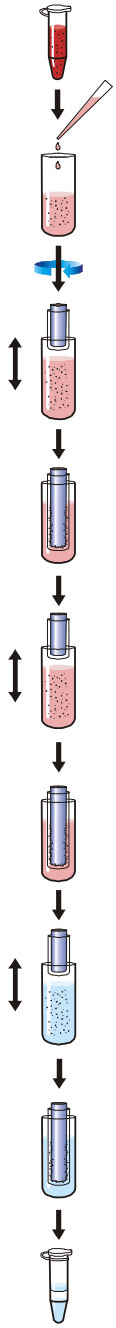
Add the required amount of dd-H_2O (see Kit Contents, page 3) to the reaction tube containing **Proteinase K**. Vortexing for 5 sec and store diluted **Proteinase K** at -20℃.

15 DNA-extractions:
Dilute **Proteinase K** by addition of 0.5 ml ddH_2O, mix thoroughly and store like described on page 3.
Add 7.5 ml of 96-100% ethanol to the bottle **Wash Buffer I**.
Add 42 ml of 96-100% ethanol to the bottle **Wash Buffer II.**
Mix thoroughly and keep the bottle always firmly closed!
75 DNA-extractions:
Dilute **Proteinase K** by addition of 2 ml of ddH_2O, mix thoroughly and store like described on page 3.
Add 30 ml of 96-100% ethanol to the bottle **Wash Buffer I**.
Add 105 ml of 96-100% ethanol to the bottle **Wash Buffer II**.
Mix thoroughly and keep the bottle always firmly closed!
Divide the **SNAP Solution** according your need.

Reagents and equipment to be supplied by user

- measuring cylinder (250 ml)
- pipette and pipette tips
- disposable gloves
- reaction tubes (1.5 ml / 2.0 ml)
- dd-H_2O
- vortexer
- 96-100% ethanol

Scheme of the InviMag® Blood DNA Mini Kit/ KFmL



Please carefully read the protocols before starting a run

- If necessary add required amount of dd-H_2O or 1x PBS to adjust the sample volume to 200 µl.
- Transfer 200 µl sample into a 1.5 ml reaction tube (not provided)
- Add 200 µl **Lysis Buffer A** and 20 µl **Proteinase K**.
- Mix by pipetting up and down or by moving up and down the magnetic rods
- Incubate the samples at 56℃ for 10 min while shaki ng
- During lysis, prefill the KingFisher Tube strip(s) with the required buffers and appropriate volumes

Tube A: Add 400 µl **Binding Buffer B6** and 20 µl **SNAP Solution**
Tube B: Add 800 µl **Wash Buffer I**.
Tube C: Add 800 µl **Wash Buffer II**.
Tube D: Add 800 µl **Wash Buffer II**.
Tube E: Add 200 µl **Elution Buffer D**.

Addition of 400 µl **Binding Buffer B6** and 20 µl **SNAP Solution** with the lysate

DNA binds to magnetic particles

Magnetic particle separation

3x Washing of the particle fixed DNA (3x 800 µl)

Magnetic particle separation

Elution of genomic DNA in 200 µl Elution Buffer D

Magnetic Separation

Pure DNA

Protocol 1: Isolation of genomic DNA from up to 200 µl of whole blood or 1–30 µl buffy coat

Please read the instructions carefully and conduct the prepared procedure.

Important Note: *The protocol has been optimized for the isolation of genomic DNA from 200 µl of whole blood or 30 µl buffy coat. For samples with a smaller volume than 200 µl, please fill up to a total volume of 200 µl with distilled water or 1x PBS.*

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

1. Transfer 200 µl of whole blood or 30 µl buffy coat into a 1.5 ml reaction tube. If the sample volume is lower than 200 µl, equilibrate with sterile water or 1x PBS.
2. Add 200 µl **Lysis Buffer A** and 20 µl **Proteinase K**.

Important Note: *Vortex the sample for 10 s ! An incomplete mixing will reduce the quality and yield of the isolated DNA.*

3. Incubate the sample at 56°C for 10 min while continuously shaking.
4. During lysis, prefill all tubes of the KingFisher tube strips with the required buffers and appropriate volumes (see "Starting a Run", page 16).
5. After lysis, transfer the sample(s) carefully into the Tube A of the corresponding KingFisher tube strip prefilled with 400 µl **Binding Buffer B6** and 20 µl **SNAP Solution**

Important Note: *Vortex the **SNAP Solution** vigorously before use !*

6. Continue with "Starting a run" on page 16

Protocol 2: Isolation of genomic DNA from up to 25 µl of non mammalian blood

Please read the instructions carefully and conduct the prepared procedure.

Important Note: *For samples which have a smaller volume than 200 µl please adjust to a total volume of 200 µl using 1x PBS or distilled water.*

If bird (e. g. chicken) or fish blood should be used that contain nucleated erythrocytes, the use of only 10-15 µl of starting material is recommended.

I. Sample Lysis

1. Transfer max. 25 µl of non-mammalian blood (*not heparin stabilized*) into a 1.5 ml reaction tube. Adjust the sample volume to 200 µl using 1x PBS or distilled water.
2. Add 200 µl **Lysis Buffer A** and 20 µl **Proteinase K**.

Important Note: *Vortex the sample for 10 s! An incomplete mixing step will reduce quality and yield of the isolated DNA.*

3. Incubate the sample at 56°C for 25 min while continuously shaking.
4. During lysis, prefill all tubes of the KingFisher mL with required buffers and appropriate volumes (see "Starting a Run", page 16).
5. After lysis, transfer the sample carefully into Tube A of the KingFisher tube strip prefilled with 400 µl **Binding Buffer B6** and 20 µl **SNAP Solution** (see "Starting a Run", page 16).

Protocol 3: Isolation of genomic DNA from CSF and bone marrow

Please read the instructions carefully and conduct the prepared procedure.

Important Note: *For samples which have a smaller volume than 200 µl please adjust to a total volume of 200 µl using 1x PBS or distilled water*

Preparation of the starting material:

Fresh material:

- 1–200 µl fresh cerebrospinal fluid
- 1-20 µl bone marrow

Dried material (for example on hematological slides):

- Moisten the dried material with a drop of PBS.
- Add 180 µl PBS to a 1.5 ml microcentrifuge tube (not provided).
- Scrape cytological material into the microcentrifuge tube using the edge of a clean slide.
- Dissolve the resulting sludge by pipetting up and down.

I. Sample Lysis

1. Transfer the starting material into a 1.5 ml reaction tube. If the sample volume is lower than 200 µl, adjust with 1 x PBS Buffer or distilled water.
2. Add 200 µl **Lysis Buffer A** and 20 µl **Proteinase K**.

Important Note: *Vortex the sample for 10 s! An incomplete mixing will reduce quality and yield of the isolated DNA.*

3. Incubate the sample at 56℃ for 20 min while con tinuously shaking.
4. During lysis, prefill all tubes of the KingFisher mL with needed buffers and appropriate volumes (see “Starting a Run”, page 16).
5. After the lysis transfer the sample carefully into the Tube A of the KingFisher tube strip prefilled with 400 µl **Binding Buffer B6** and 20 µl **SNAP Solution** (see “Starting a Run”, page 16).

Important Note: *Vortex the **SNAP Solution** vigorously before use !*

Protocol 4: Isolation of genomic DNA from swabs or rinsed liquid from swabs

Please read the instructions carefully and conduct the prepared procedure.

Important Note: *For samples which have a smaller volume than 200 µl, please fill up to a total volume of 200 µl with 1x PBS or distilled water. If chicken or fish blood is used a final sample volume of 10 µl is recommended.*

Fresh or dried swabs

I. Sample Lysis

1. Add 180 µl PBS-Buffer or distilled water to 1.5 ml reaction tube (not provided). Transfer the swab into the tube and incubate for 3 min. Afterwards add 200 µl **Lysis Buffer A** and 20 µl **Proteinase K**. Mix by pipetting up and down (5 times).

2. Incubate the reaction tube at 56°C for 20 min while continuously shaking on a thermomixer.

***Important Note**: To get maximum yield of DNA, it is essential to leave the swab inside the tube during the complete lysis time. It is possible to cut-off the shaft of the swab to allow closing of reaction tube cap. The removing of the swab from the reaction tube ahead of time will lead to a dramatically reduced final yield!*

3. During lysis, prefill all tubes of the KingFisher mL with required buffers and appropriate volumes (see "Starting a Run", page 16).
4. After lysis, carefully squeeze out the swab inside the tube wall and discard the swab. Transfer the sample carefully into Tube A of the KingFisher tube strip prefilled with 400 µl **Binding Buffer B6** and 20 µl **SNAP Solution** (see "Starting a Run", page 16).

***Important Note:** Vortex the **SNAP Solution** vigorously before use !*

Swabs delivered in transportation media

***Important:** If the swab is delivered in stabilization media, ensure that these media are compatible with the STRATEC Molecular chemistry. For information contact STRATEC Molecular + 49 30 9489 2907.*

1. Transfer 200 µl of the transportation media into a 1.5 ml reaction tube. If the sample volume is lower than 200 µl, equilibrate with 1x PBS or distilled water.
2. Add 200 µl **Lysis Buffer A** and 20 µl **Proteinase K**.

***Important Note:** Vortex the sample for 10 s! An incomplete mixing will reduce quality and yield of the isolated DNA.*

3. Incubate the sample at 56°C for 20 min while continuously shaking.
4. During lysis, prefill all tubes of the KingFisher mL with required buffers and appropriate volumes (see "Starting a Run", page 16).
5. After the lysis transfer the sample carefully into the Tube A of the KingFisher tube strip prefilled with 400 µl **Binding Buffer B6** and 20 µl **SNAP Solution** (see "Starting a Run", page 16).

Use of the rinsed liquid from the swab

1. If the swab is delivered without transport media, rinse each swab in a 1.5 ml reaction tube with 200–500 µl cooled disatilled water or 1x PBS. Mix for several minutes by shaking.
2. Follow the protocols for sample lysis found in the previous section "Swab delivered in transportation media" and use 200 µl of the rinsed liquid as starting material.

Starting a run - Preliminary Steps to process the sample onto the KingFisher System

Important: *For working with the KingFisher mL instrument, please read carefully the manufacturer's documents!*

1. During the sample lysis prefill the tubes of the KingFisher tube strips with the following buffers and appropriate volumes:

Tube A: Add 400 µl **Binding Buffer B6** and 20 µl **SNAP Solution.** After lysis, transfer the sample into tube A of the KFmL stripe

Important: *Mix the bottle with **SNAP Solution** carefully by vigorously shaking or vortexing !*

Tube B: Add 800 µl **Wash Buffer I.**

Tube C: Add 800 µl **Wash Buffer II.**

Tube D: Add 800 µl **Wash Buffer II.**

Tube E: Add 200 µl **Elution Buffer D.**

2. Place the prefilled KingFisher tube strips with the tube tray into the KingFisher system

3. Place the KingFisher tip combs into the slots of the instrument!

4. Choose KFmL assay file "**InviMag Blood DNA KFmL**" and press the "START" button

The following extraction steps run automatically on the KFmL system:

Binding of the DNA
Automatically sample mixing for 5 minutes. MAP separation. Moving of the MAP into well B.

First Washing
Automatically sample mixing for 90 s. MAP separation. Moving of the MAP into well C.

Second Washing
Automatically sample mixing for 1 min. MAP separation. Moving of the MAP into well D.

Third Washing and Drying
Automatically sample mixing for 1 min. MAP separation. Drying the MAP outsight the Tube for 5 min. Moving of the MAP into the well E.

Elution of the DNA
Incubation of the MAP into the Tube E for 15 minutes by mixing. MAP separation. The MAP will then be automatically removed into well D (disposal).

Important Note: *After finishing the extraction protocol, the Tube E contains the extracted genomic DNA Store the DNA under adequate conditions. We recommend to transfer the extracted DNA into 1.5 ml reaction tubes for further storage and freeze the DNA at –20°C.*

If the extracted DNA contains carryover of magnetic particles, transfer the DNA into a 1.5 ml reaction tube, centrifuge at maximum speed for 1 min and transfer the DNA-containing supernatant into a new tube.

For self programming the KFmL system

[PROTOCOL PROPERTIES]
Name = InviMag Blood DNA Mini Kit/ KFmL
Protocol template version = 3.1
Instrument type = KFmL
Description = KFmL protocol for isolation of genomic DNA from blood samples with the InviMag Blood DNA Mini Kit/ KFmL

[PLATE LAYOUTS]

Binding plate
Plate type = KingFisher tub strip 1000µl
Plate change message = Insert Bind plate
- volume = 200, name = Sample
- volume = 200, name = Lysis Buffer A
- volume = 20, name = Proteinase K

Washing plate_1
Plate type = KingFisher tub strip 1000µl
Plate change message = Insert Wash 1
- volume = 800, name = Wash buffer I

Washing plate_2
Plate type = KingFisher tub strip 1000µl
Plate change message = Insert Wash 2
- volume = 800, name = Wash buffer II

Washing plate_3
Plate type = KingFisher tub strip 1000µl
Plate change message = Insert Wash 3
- volume = 800, name = Wash buffer II

Elution plate
Plate type = KingFisher tub strip 1000µl
Plate change message = Insert Elution
- volume = 200, name = Elution buffer D

[STEPS]
Binding
Plate: Blood Mini (A)

Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Fast

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:05:00
Mixing speed: Slow

End of step:
Postmix: No
Collect count: 4
Collect time [s]: 3

Washing_1
Plate: Blood Mini (B)

Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Fast

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:01:30
Mixing speed: Fast

End of step:
Postmix: No
Collect count: 3
Collect time [s]: 2

Washing_2
Plate: Blood Mini (C)

Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Fast

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:01:00
Mixing speed: Fast

End of step:
Postmix: No
Collect count: 3
Collect time [s]: 2

Washing_3
Plate: Blood Mini (D)

Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Fast

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:01:00
Mixing speed: Fast
End of step:
Postmix: No
Collect count: 3

Collect time [s]: 2

Drying
Plate: Washing Plate 4

Dry time [hh:mm:ss]: 00:05:00*
Tip position: Outside well / tube

Elution
Plate : Blood Mini (E)

Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:00:10
Release speed: Medium

Mixing/pause parameters:
Pause for manual handling: No
Mixing time [hh:mm:ss]: 00:10:00
Mixing speed: Slow

End of step:
Postmix: No
Collect count: 4
Collect time [s]: 3

Remove_Beads
Plate: Blood Mini (D)

Release time [hh:mm:ss]: 00:00:30
Release speed: Fast

Troubleshooting

Problem	Probable cause	Comments and suggestions
low amount of extracted DNA	insufficient lysis	increase lyses time. Reduce amount of starting material
	incomplete elution	take higher volume of **Elution Buffer D**, be sure you pipet the elution Buffer D with the right amount to the right position
	low amount of SNAP Solution	mix SNAP Solution thoroughly before pipetting to the KingFisher tube
low concentration of extracted DNA	too much **Elution Buffer D**	elute the DNA with lower volume of **Elution Buffer D**
	incorrect storage of starting material	ensure that the storage of starting material was correctly Avoid thawing of the material
degraded or sheared DNA	incorrect storage of starting material	ensure that the storage of starting material was correctly Avoid thawing of the material
	old material	ensure that the starting material is fresh or stored under appropriate condition (for long time storage at –20℃)! avoid thawing and freezing of the material 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 removing of ethanol
	salt carryover during elution	check up the **Wash Buffers** for salt precipitates. If there are any precipitates, solve these precipitates by careful warming ensure that the **Wash Buffers** are 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 down at full speed for 1 min and transfer supernatant to a new tube

Appendix

KingFisher Software 3.1

The KingFisher Software 3.1 was used to create assay files for the KFmL, KF96 and KFflex96 instruments. The respective assay file can either be transferred onto the KingFisher workstation or be started directly from within the BindIt software. Keep in mind that directly run assay files are not stored in the workstation memory!

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 3.1 for assay development as this software version includes the correct adjustments for this plate. It is highly recommended to use Thermo Microtiter Deep Well plates with KF96 / KFflex96 workstations to ensure the best purification result.*

PC requirements for KingFisher Software 3.1

PC requirements	
Interface	Serial communication port via a RS-232 full duplex interface
Supported operating systems	Microsoft Windows 2000 Microsoft Windows XP Professional
Disk space	500 MB free disk space
Processor	Intel Pentium ≥ 700 MHz recommended
Memory	220 MB RAM recommended
Serial ports available	1
Pointing device	Mouse or equivalent is necessary
CD-ROM drive	1
Monitor / color settings	SVGA monitor with at least 1024 x 768 resolution and at least a 16-bit color environment
Service packs installed	Microsoft Windows 2000: Service Pack 4 (or greater) Microsoft Windows XP Professional: Service Pack 2 (or greater)
Browser	Microsoft Internet Explorer 6.0 (or greater) installed

If you do not have the correct Service Packs installed, you can download them from the Microsoft web pages: http://www.microsoft.com/

General notes on handling DNA

Starting material
This kit is designed for extraction of DNA from blood, but even human blood is different between individuals depending on age, health, and conditions of life. If you are using blood from animals keep in mind that lyses conditions of blood differ depending on the species. Also remember that non-mammalian blood contains erythrocytes with nuclei. So for special applications adaptation of starting volumes and lyses time may be recommended.

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 necessary to ensure compatibility with various downstream applications. Damaged DNA could perform poorly in applications such as genomic Southern blotting, long-template PCR.

Storage of DNA
A working stock of DNA can be stored at 2–4˚C for several weeks. For long-term storage DNA should be stored at -20˚C, but storing at -20℃ can cause shearing, particularly if the DNA is exposed to repeated freeze-thawing cycles.

Note that the solution in which the nucleic acid is eluted in, will affect the 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 over drying 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.
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.

DNA Yield
The amount of purified DNA from the whole blood, depends on the leucocytes content, sample source, transport, storage, and age. Various different primary tubes and anticoagulants (except heparin) can be used to collect blood samples for the **Invisorb®** procedure.

Order information

Product	Package Size	Order No
InviMag® Blood DNA Mini Kit/ KFmL	15 preparations	2431110100
InviMag® Blood DNA Mini Kit/ KFmL	75 preparations	2431110200
InviMag® Blood DNA Mini Kit/ KF96	1 x 96 preparations	7431300100
InviMag® Blood DNA Mini Kit/ KF96	5 x 96 preparations	7431300200
Invisorb® Spin Blood Mini Kit	50 preparations	1031100200
Invisorb® Spin Blood Mini Kit	250 preparations	1031100300
Invisorb® Spin Blood Midi Kit	50 preparations	1031110300
Invisorb® Spin Blood Midi Kit	250 preparations	1031110500
Invisorb® Spin Blood Maxi Kit	50 preparations	1031120200
Invisorb® Spin Blood Maxi Kit	250 preparations	1031120300
Invisorb® Blood Universal Kit	50 ml	1031150100
Invisorb® Blood Universal Kit	500 ml	1031150200
Invisorb® Blood Mini HTS 96 Kit/ C	4 x 96 preparations	7031300300
Invisorb® Blood Mini HTS 96 Kit/ C *using a centrifuge*	24 x 96preparations	7031300400
Invisorb® Blood Midi HTS 96 Kit/ C	4 x 96 preparations	7031700300
Invisorb® Blood Midi HTS 96 Kit/ C *using a centrifuge*	24 x 96preparations	7031700400
Invisorb® Blood Mini HTS 96 Kit/ V	4 x 96 preparations	7031310300
Invisorb® Blood Mini HTS 96 Kit/ V *using a vacuum manifold*	24 x 96 preparations	7031310400
Invisorb® Blood Mini HTS 96 Kit/ R	4 x 96 preparations	7131300300
Invisorb® Blood Mini HTS 96 Kit/ R *using a robotic station*	24 x 96preparations	7131300400
Invisorb® Blood Mini HTS 96 Kit/ X	4 x 96 preparations	7131310300
Invisorb® Blood Mini HTS 96 Kit/ X *using the X-tractor Gene™, Corbett Robotics*	24 x 96 preparations	7131410400
Invisorb® Blood Mini HTS 96 Kit/ ep	4 x 96 preparations	7131320300
Invisorb® Blood Mini HTS 96 Kit/ ep *using the epMotion® 5075 VAC, Eppendorf*	24 x 96 preparations	7131320400

Single Components for InviMag® Blood DNA Mini Kit

Lysis Buffer A	30 ml	7431301100
SNAP Solution	1.5 ml	7431305200
Binding Buffer B6	30 ml	7431302100
Elution Buffer D	30 ml	7431304000
Wash Buffer I	30 ml	7431303300
Wash Buffer II	60 ml	7431303400