Instruction for InviMag® Blood DNA Mini Kit/ KF96/ KFflex96

The **InviMag® Blood DNA Mini Kit/ KF96/ KFflex96** combines the advantages of the innovative Invisorb® technology with easy handling of magnetic particles in combination with KingFisher instruments for a very efficient and reliable isolation of nucleic acids with a high purity.

The **InviMag® Blood DNA Mini Kit/ KF96/ KFflex96** is the ideal tool for a semi-automated isolation and purification of pure DNA (genomic and mitochondrial) from large number of 200 µl whole blood samples (stabilized with EDTA, citrate ***but not heparin***), buffy coat, non-mammalian blood, cerebrospinal fluid (CSF), bone marrow, and swabs in a 96 well format. The kit has been designed for an optimal use on the KF96 and/or KFflex96 workstations from Thermo Scientific. The interplay of the DNA extraction and purification chemistry provided by the **InviMag® Blood DNA Mini Kit/ KF96/ KFflex96** in combination with the corresponding KingFisher instrument was intensely tested and validated.

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

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

The kit is neither validated for the isolation of genomic DNA from cultured or isolated cells, from tissues, blood cards, dried blood stains, urine nor from stool samples, bacteria, fungi, parasites, or the purification of total RNA. The application of the kit for isolation and purification of viral DNA has not been evaluated.

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

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Store the **SNAP Solution** at 2-8°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)!

	1 x 96 extractions	**5 x 96 extractions**
Catalogue Number	7431300100	7431300200
Lysis Buffer A	30 ml	120 ml
Binding Buffer B6	50 ml	220 ml
Proteinase K (working solution)	2 ml	10.5 ml
SNAP Solution	2 x 1.1 ml	10.5 ml
Wash Buffer I	2 x 30 ml (final volume 2 x 60 ml)	2 x 125 ml (final volume 2 x 250 ml)
Wash Buffer II	60 ml (final volume 200 ml)	6 x 45 ml (final volume 6 x 150 ml)
Elution Buffer D	30 ml	120 ml
2.0 ml Deep Well Plate	5	25
KF 96 Tip Comb for DW magnets	1	5
200 µl Elution Plate*	2	10
1.5 ml Receiver Tubes	2 x 50	10 x 50
Sealing Foils	2	10
Manual	1	1
Initial steps	Add 30 ml of 96-100% ethanol to each bottle **Wash Buffer I,** mix thoroughly and keep the bottle always firmly closed! Add 140 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and keep the bottle always firmly closed! Dilute **Proteinase K** by addition of 2 ml of ddH_2O, mix thoroughly and store like described below.	Add 125 ml of 96-100% ethanol to each bottle **Wash Buffer I**, mix thoroughly and keep the bottle always firmly closed! Add 105 ml of 96-100% ethanol to each bottle **Wash Buffer II,** mix thoroughly and keep the bottle always firmly closed! Dilute **Proteinase K** by addition of 10.5 ml of ddH_2O, mix thoroughly and store like described below. Divide the SNAP Solution according your need.

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

Kit contents of InviMag® Blood DNA Mini Kit/ KF96/ KFflex96 /wp

Store the **SNAP Solution** at 2-8°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)!

	1 x 96 extractions	**5 x 96 extractions**
Catalogue Number	7431300150	7431300250
Lysis Buffer A	30 ml	120 ml
Binding Buffer B6	50 ml	220 ml
Proteinase K (working solution)	2 ml	10.5 ml
SNAP Solution	2 x 1.1 ml	10.5 ml
Wash Buffer I	2 x 30 ml (final volume 2 x 60 ml)	2 x 125 ml (final volume 2 x 250 ml)
Wash Buffer II	60 ml (final volume 200 ml)	6 x 45 ml (final volume 6 x 150 ml)
Elution Buffer D	30 ml	120 ml
1.5 ml Receiver Tubes	2 x 50	10 x 50
Sealing Foils	2	10
Manual	1	1
Initial steps	Add 30 ml of 96-100% ethanol to each bottle **Wash Buffer I,** mix thoroughly and keep the bottle always firmly closed! Add 140 ml of 96-100% ethanol to the bottle **Wash Buffer II**, mix thoroughly and keep the bottle always firmly closed! Dilute **Proteinase K** by addition of 2 ml of ddH_2O, mix thoroughly and store like described below.	Add 125 ml of 96-100% ethanol to each bottle **Wash Buffer I**, mix thoroughly and keep the bottle always firmly closed! Add 105 ml of 96-100% ethanol to each bottle **Wash Buffer II,** mix thoroughly and keep the bottle always firmly closed! Dilute **Proteinase K** by addition of 10.5 ml of ddH_2O, mix thoroughly and store like described below. Divide the SNAP Solution according your need.
Plastic to be supplied by user (see order information)		
2.0 ml Deep Well Plate	5	25
KF 96 Tip Comb for DW magnets	1	5
200 µl Elution Plate*	2	10

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

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/ KF96/ KFflex96**, 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℃. 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 2-8℃.

Wash Buffers: Wash Buffers charged with ethanol should be stored at room temperature* and should be well sealed. If any precipitates are 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/ KF96/ KFflex96** 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/ KF96/ KFflex96** 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/ KF96/ KFflex96** 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/ KF96/ KFflex96** has been designed for semi-automated extraction and purification of total (genomic and mitochondrial) DNA from 8-96 whole blood or blood related samples using magnetic beads and the KF96 or KFlex96 instrument. The nucleic acid isolation protocol is suitable for routinely walk-away automated preparation of DNA from fresh or frozen whole blood samples, buffy coats, non-mammalian bloods, cerebrospinal fluids (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 10). 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/ KF96/ KFflex96** in different package sizes.

The procedure of the **InviMag® Blood DNA Mini Kit/ KF96/ KFflex96** 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 with 1x PBS prior to the start of an isolation protocol.

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.

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 genomic DNA from cultured or isolated cells, from tissue, blood cards, dried blood stains, urine nor from stool sample, bacteria, fungi, parasites, or the purification of total RNA. The application of the kit for isolation and purification of viral DNA has not been evaluated.

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/ KF96/ KFflex96** 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/ KF96/ KFflex96** to which they apply, are listed.

Wash Buffer I contains guanidine thiocyanate which is an irritant.

Lysis Buffer A **Binding Buffer B6**

danger **danger**

H-319 P305-351-338 **H-225 H319 H-336 P210-233-305-351-338**

Proteinase K **Wash Buffer I**

danger **warning**

H315-319-334-335 P280-305-351-338-310-405 **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 language 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/ KF96/ KFflex96

The **InviMag® Blood DNA Mini Kit/ KF96/ KFflex96** procedure is 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 96 well format using magnetic beads and the KF96 or KFflex96 instrument.

Starting Material	Yield	Time for	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	3–12 µg, depends on the blood sample (storage and source)	about 45 min	A_{260}:A_{280}: 1.8-2.0

The semi-automated DNA isolation process is based on the interaction of nucleic acids with coated magnetic particles under adapted buffer conditions. After lysis, KingFisher instrument performs all steps of the DNA purification procedure automatically without any user intervention. The procedure requires only minimal interaction by the user, thus allowing safe handling of potentially infectious samples. 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-elution. The volume of buffers and other liquids necessary for DNA isolation is reduced to a minimum. Eliminating most of the direct liquid handling and increasing the automation level results in a fast, reliable and robust technique.

After a sample specific cell lysis on the workstation, using the **Lysis Buffer A** and **Proteinase K** optimal binding conditions are adjusted by addition of **Binding Buffer B6.** The released DNA binds to the simultaneously added magnetic particles and is separated from solution by the magnetic rods controlled by the KingFisher machine. Subsequent to four washing steps of the particle bound nucleic acids, the DNA is eluted in **Elution Buffer D**.

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

- PCR*, Real-time PCR, PCR, qPCR
- Restriction Enzyme Digestion
- HLA Typing
- Southern Blot.

The **InviMag® Blood DNA Mini Kit/ KF96/ KFflex96** is supplied with a comprehensive manual describing four protocols (page 13-14) for DNA purification from different sources. For the semi-automated isolation of genomic DNA from 200 µl blood using magnetic particles in a single well format for up to 15 samples per run, STRATEC Molecular offers the **InviMag® Blood DNA Mini Kit/ KFmL** for use on a KingFisher mL instrument and for 1 ml whole blood the **InviMag® Blood DNA Mini Kit/ KFflex24.**

* 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 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 robotic station (see “Ordering information”, see page 24).

For further information please contact: Phone: +49 (0) 30 9489 2894, 2910 in Germany and from foreign countries Phone: +49 (0) 30 9489 2907 or your local distributor.

Product validation

PCR inhibitor and cross contamination test

To maximize the detection of any potential contamination event, positive and no template controls (NTCs) were arranged in alternating wells (in a “chessboard” pattern ***Fig. 1***). Out of those samples ***Fig. 2*** shows a real-time PCR run of the extracted DNA. PCRs were done with a GAPDH Primer set in an in-house SyBr Green assay on a Corbett Rotor Gene 3000.

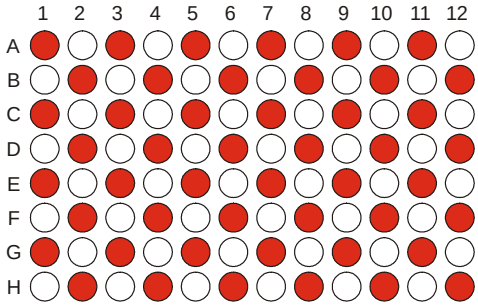


Fig. 1 **‘Chessboard Pattern’** utilized for the cross contamination analysis test. Samples (red) and NTC (white) arranged in alternating wells.

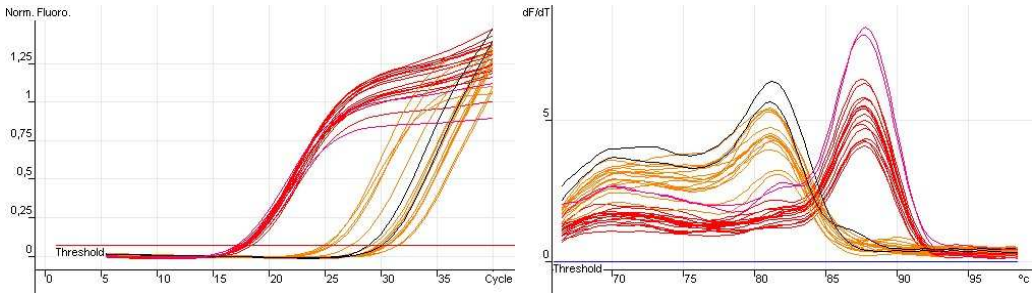
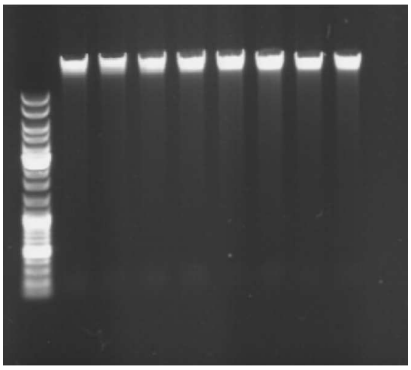


Fig. 2 Real-time PCR results from samples (red) and no samples controls (yellow) arranged in Chessboard. NTC (black) and PTC (pink) are also shown.

Reproducibility



DNA was isolated from 200 µl pooled blood from transfusion center (incl. 20% stabilization solution)

The ratio $A_{260/280}$ is between 1,86-1,89.

The yield is about 3.82 +/- 0,21.

Sampling and storage of 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) can be stored at room temperature for 2-3 hours, for short time storage (up to 24 h) samples may be stored at 4°C. For long term s torage, we recommend freezing samples at -20°C or -80°C. Multiple thawing and freezing cycle s 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/ KF96/ KFflex96** procedure.

Buffy coat is a whole-blood fraction of enriched leukocyte cells. To prepare and extract a buffy coat layer 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 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 DNA purification system.

CSF (cerebrospinal fluid) and bone marrow:
Best results are obtained with fresh material than can be stored for 2-3 h at 4°C for short-term storage. For long-term storage freeze the sample at -20°C. However, the sample will often be dried. Dried samples have to be stored cooled 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 swab samples are often characterized by isolation of apoptotic DNA (visible on agarose gel as a 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/ KF96/ KFflex96** procedure comprises following steps:

- lysis of blood cells and protein digestion
- binding the genomic DNA to the magnetic beads
- washing the bead bound DNA and elimination of ethanol
- elution of genomic DNA.

After lysis, the DNA binds to the magnetic beads whereas contaminations and enzyme inhibitors are efficiently removed during the following four washing steps and highly purified DNA is eluted in Elution Buffer D.

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

Procedure

Lysis

Samples with a volume lower than 200 µl should be adjusted to 200 µl using 1x PBS or distilled water before starting the protocol. For optimal results, samples must be equilibrated to room temperature before lysis.

Samples are lysed under denaturing conditions at elevated temperatures in the presence of **Lysis Buffer A** and **Proteinase K**. In case of large number of samples 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 genomic DNA

After adding **Binding Buffer B6** and **SNAP Solution**, 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 from the beads using 150 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 Quality

The amount of purified DNA in the **InviMag® Blood DNA Mini Kit/ KF96/ KFflex96** procedure from whole blood depends on the leucocytes content, the sample source, transport, storage, and 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 get from the **InviMag® Blood DNA Mini Kit/ KF96/ KFflex96** is 3-8 µg DNA. If the whole blood sample is mixed with anticoagulant containing buffer solutions 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 notes

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, since their use 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, bring all reagents to room temperature. Where necessary, gently mix and redissolve any precipitates by incubation at 30°C. Swirl gently to avoid foaming.

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

Add the required amount of ddH_2O (see "Kit contents", page 4) to reaction tube with **Proteinase K**. Vortex for 5 sec and store diluted **Proteinase K** at -20°C.

1 x 96 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 140 ml of 96-100% ethanol to the bottle **Wash Buffer II.**
Mix thoroughly and keep the bottle always firmly closed!
5 x 96 DNA-extractions:
Dilute **Proteinase K** by addition of 10.5 ml of ddH_2O, mix thoroughly and store like described on page 3.
Add 125 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 needs.

Reagents and equipment to be supplied by user

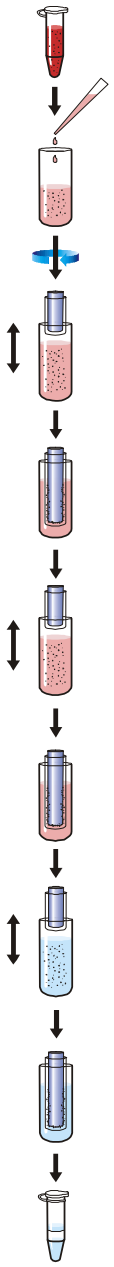
- measuring cylinder (250 ml)
- pipette and pipette tips
- disposable gloves
- reaction tubes (1.5 ml or 2.0 ml)
- dd-H_2O
- vortexer
- 96-100% ethanol

Scheme of the InviMag® Blood DNA Mini Kit/ KF96/ KFflex 96

Please read protocols prior the start of the preparation carefully

Add required amount of dd-H_2O or 1x PBS to adjust the sample volume to 200 µl. Transfer the sample into a well of the Binding Plate. Prefill all remaining plates with required buffers and appropriate volumes.

Tip Plate:	Place the KF96 Tip Comb for DW magnets in one 200 µl **Elution Plate***
Binding Plate:	Add 200 µl **sample**, 20 µl **Proteinase K** and 200 µl **Lysis Buffer A**. After lysis, a pause step occurs and 400 µl **Binding Buffer B6** and 20 µl **SNAP Solution** have to be added to the lysate
Washing Plate_1:	Add 500 µl **Wash Buffer I** to a 2.0 ml Deep Well Plate
Washing Plate_2:	Add 500 µl **Wash Buffer I** to a 2.0 ml Deep Well Plate
Washing Plate_3:	Add 800 µl **Wash Buffer II** to a 2.0 ml Deep Well Plate
Washing Plate_4:	Add 800 µl **Wash Buffer II** to a 2.0 ml Deep Well Plate
Elution Plate:	Pipet 150 µl **Elution Buffer D** to a 200 µl Elution Plate



200 µl sample is mixed with 200 µl **Lysis Buffer A** in presence of 20 µl **Proteinase K.**

Incubation at 60℃ for 10 min

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

DNA binds to magnetic particles

Magnetic separation

Washing of the particle fixed genomic DNA
2x 500 µl **Wash Buffer I**
2x 800 µl **Wash Buffer II**

Magnetic separation

Elution of genomic DNA in 150 µl **Elution Buffer D**

Magnetic separation and bead removal

Pure DNA

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

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

Please read the instructions carefully and conduct the prepared procedure.

1. Transfer 200 µl of whole blood or 30 µl buffy coat into the cavities of the Binding Plate. If the sample volume is lower than 200 µl, adjust either with 1x PBS or distilled water.
2. Add 200 µl **Lysis Buffer A** and 20 µl **Proteinase K** to sample containing cavities of the Binding Plate.
3. Proceed with prefilling of the plates and preparation of the instrument (see “Starting a Run”, 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 fill up to a total volume of 200 µl with 1x PBS or distilled water prior to start.*

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

1. Transfer max. 25 µl of non-mammalian blood (*not heparin stabilized*) into the cavities of the Binding Plate. Adjust the sample volume to 200 µl with 1x PBS or distilled water.
2. Add 200 µl **Lysis Buffer A** and 20 µl **Proteinase K** to sample containing cavities of the Binding Plate.
3. Proceed with prefilling of the plates and preparation of the instrument (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 fill up to a total volume of 200 µl with 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.

1. Transfer the starting material into the cavities of the Binding Plate. Adjust the sample volume to 200 µl with 1x PBS or distilled water.

2. Add 200 µl **Lysis Buffer A** and 20 µl **Proteinase K** to sample containing cavities of the Binding Plate.

3. Proceed with prefilling of the plates and preparation of the instrument (see "Starting a Run", page 16)

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

Please read the instructions carefully and conduct the prepared procedure.

Dried swabs:

If the swab is delivered without transportation media, rinse the swab in a 1.5 ml reaction tube filled with 200-500 µl cooled water or 1x PBS (4℃) . Mix for several minutes by shaking and continue with step 1 (see below)

Rinsed swabs:

1. Squeeze out the swab inside the wall of the transportation tube and discard it.

2. Transfer 200 µl of the transportation media into a 1.5 ml reaction tube. If the sample volume is lower than 200 µl, fill up with 1x PBS or distilled water.

3. Add 200 µl **Lysis Buffer A** and 20 µl **Proteinase K** to sample containing cavities of the Binding Plate.

4. Proceed with prefilling of the plates and preparation of the instrument (see "Starting a Run", page 16)

Starting a run on a KF96 / KFflex96 instrument

Important: *For working with the KingFisher instruments please carefully read the manufacturer's documents before use!*

1. Turn on the KF96 or KFflex96 instrument

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

2. Prefill all Deep Well Plates with the appropriate buffers and volumes indicated below

3. **Tip Plate**: Place the provided Tip Comb for DW magnets on a 200 µl Elution Plate.

Binding Plate: Add 200 µl sample, 20 µl Proteinase K and 200 µl Lysis Buffer A. After lysis, a pause step occurs and 400 µl **Binding Buffer B6** and 20 µl **SNAP Solution** have to be added to the lysed sample.

Washing plate_1: Transfer 500 µl **Wash Buffer I**

Washing plate_2: Transfer 500 µl **Wash Buffer I**

Washing plate_3: Transfer 800 µl **Wash Buffer II**

Washing plate_4: Transfer 800 µl **Wash Buffer II**

Elution Plate: Transfer 150 µl **Elution Buffer D**

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

4. Choose either the KF96 protocol **"InviMag Blood DNA Mini KF96"** or the KFflex96 protocol **"InviMag Blood DNA Mini KFflex96"** depending on the used instrument and press the "START" button
5. Insert all prefilled plates into the instrument by following the specifications printed on the display and confirm every plate loading step by pressing the "START" button.
6. After all prefilled plates have been loaded, press the "START" button finally to initialize the assay file. The run will take approximately 45 min.

After lysis, a pause occurs and 400 µl **Binding Buffer B6** and 20 µl **SNA Solution** have to be added to sample containing cavities of the Binding Plate. For this purpose, the instrument will be paused automatically after lysis and a message is shown on the KingFisher display that the beads and binding buffer have to be added. The plate is transported to the loading position. After adding both reagents, reinsert the plate into the instrument and confirm this step by pressing "START" again. The machine will continue with the extraction without any further user interaction. Watch out that the orientation of the plate inside the turntable is correct

The following extraction steps run automatically on the KingFisher instrument

Lysis of the blood cells

Automatically sample mixing for 10 min at 60℃.

Adjustment of Binding condition

Magnetic Beads (SNAP Solution) and Binding Buffer B6 are added to the lysed sample

Binding of the DNA

Automatically sample mixing for 5 min. SNAP separation. Moving of the SNAP into the Washing Plate_1.

First Washing

Automatically sample mixing for 1.5 min. SNAP separation. Moving of the SNAP into the Washing Plate_2.

Second Washing

Automatically sample mixing for 1.5 min. SNAP separation. Moving of the SNAP into the Washing Plate_3.

Third Washing

Automatically sample mixing for 1 min. SNAP separation. Moving of the SNAP into the Washing Plate_4.

Fourth Washing and Drying

Automatically sample mixing for 1 min. SNAP separation. Drying the SNAP outsight the plate for 5 min. Moving of the SNAP into the Elution Plate.

Elution of the DNA

Incubation of the SNAP into the Elution Plate for 10 min by mixing at 60℃. SNAP separation.

The SNAP will then be automatically removed into the wells of Washing Plate_4 (disposal).

Important Note: 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 a 1.5 ml reaction tube and freeze the DNA at -20℃.

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

For self programming the KF96 / KFflex96 instrument

[PROTOCOL PROPERTIES]

Name = InviMag Blood DNA Mini Kit /KF96 /KFflex96
Protocol template version = 3.1
Instrument type = KF96 / KFflex96
Description = KF96 / KFflex96 protocol for isolation of genomic DNA from blood samples with the InviMag Blood DNA Mini Kit/ KF96/ KFflex 96.

[PLATE LAYOUTS]

Tip comb plate
Plate type = KingFisher 96 plate
Plate change message = Load tip plate

Binding plate
Plate type = Thermo DW
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 = Thermo DW
Plate change message = Insert Wash 1
- volume = 500, name = Wash buffer I

Washing plate_2
Plate type = Thermo DW
Plate change message = Insert Wash 2
- volume = 500, name = Wash buffer I

Washing plate_3
Plate type = Thermo DW
Plate change message = Insert Wash 3
- volume = 800, name = Wash buffer II

Washing plate_4
Plate type = Thermo DW
Plate change message = Insert Wash 4
- volume = 800, name = Wash buffer II

Elution plate
Plate type = KingFisher 96 plate
Plate change message = Insert Elution
- volume = 150, name = Elution buffer D

[STEPS]

Tip Plate
Pick-Up plate Tip Plate
Leave plate: Tip Plate

Lysis
Plate: Binding Plate

Beginning of step:
Precollect: No

Mixing/heating parameters:
Heating temperature [℃]: 60
Preheat: Yes
Mixing time [hh:mm:ss]: 00:10:00
Mixing speed: Medium

End of step:
Postmix: No
Collect beads: No

Add_Binding
Plate: Binding Plate

Message: Add Binding and Bead
Dispensing volume [µl]: 420
Reagent name: Binding Buffer and Beads

Binding
Plate: Binding Plate

Beginning of step:
Precollect: No
Release time [hh:mm:ss]: 00:01:00
Release speed: Fast

Mixing/heating parameters:
Heating during mixing: No
Mixing time [hh:mm:ss]: 00:05:00
Mixing speed: Slow

End of step:
Postmix: No
Collect count: 3
Collect time [s]: 1

Washing_1
Plate: Washing Plate 1

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

Mixing/heating parameters:
Heating during mixing: No
Mixing time [hh:mm:ss]: 00:01:30
Mixing speed: Fast

End of step:
Postmix: No
Collect count: 2
Collect time [s]: 1

Washing_2
Plate: Washing Plate 2

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

Mixing/heating parameters:
Heating during mixing: No
Mixing time [hh:mm:ss]: 00:01:30
Mixing speed: Fast

End of step:
Postmix: No
Collect count: 2
Collect time [s]: 1

Washing_3
Plate: Washing Plate 3

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

Mixing/heating parameters:
Heating during mixing: No
Mixing time [hh:mm:ss]: 00:01:00
Mixing speed: Medium

End of step:
Postmix: No
Collect count: 2
Collect time [s]: 1

Washing_4
Plate: Washing Plate 4

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

Mixing/heating parameters:
Heating during mixing: No
Mixing time [hh:mm:ss]: 00:01:00
Mixing speed: Medium

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 : Elution Plate

Beginning of step:
Precollect: No
Release beads: Yes
Release time [hh:mm:ss]: 00:00:30
Release speed: Medium

Mixing/heating parameters:
Heating temperature [℃]: 50
Preheat: Yes
Mixing time [hh:mm:ss]: 00:10:00
Mixing speed: Slow

End of step:
Postmix: No
Collect count: 5
Collect time [s]: 2

Remove_Beads
Plate: Washing Plate 3

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°C)!
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
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

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℃. This procedure minimizes degradation of crude DNA by limiting the activity of endogenous nucleases.

Storage of DNA

Store genomic DNA at 2-8℃. Storing genomic DNA at -20℃ may cause shearing of DNA, particularly if the DNA is exposed to repeated freeze-thawing cycles. Plasmid DNA and other small circular DNAs can be stored at 2-8℃ or at -20℃.

Drying, dissolving and pipetting DNA

Avoid overdrying genomic DNA after ethanol precipitation. It is better to air dry DNA 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 incubate the DNA in buffer overnight at 2-8℃. Minimize vortexing of genomic DNA because this can cause shearing.

Avoid vigorous pipetting. Pipetting genomic DNA through small tip openings may cause shearing or nicking. One way to decrease shearing of genomic DNA is to use special tips that have wide openings especially designed for pipetting genomic DNA. Regular pipette tips pose no problem for plasmid or other small DNA.

Order information

Product	Package size	Order-Nr.
InviMag® Blood DNA mini Kit/ KF 96	1 x 96 preparations	7431300100
InviMag® Blood DNA mini Kit/ KF 96	5 x 96 preparations	7431300200

Single components for InviMag® Blood DNA mini Kit/ KF 96

Lysis Buffer A	30 ml	7431301100
Binding Buffer B6	30 ml	7431302100
SNAP Solution	1.5 ml	7431305200
Wash Buffer I (add 30 ml ethanol)	30 ml	7431303300
Wash Buffer II (add 140 ml ethanol)	60 ml	7431303400
Elution Buffer D	30 ml	7431304000

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	Order-Nr.
InviMag® Blood DNA mini Kit/ KFmL	15 preparations	2431110100
InviMag® Blood DNA mini Kit/ KFmL	75 preparations	2431110200
Invisorb® Spin Blood mini Kit	3 preparations	1031100100
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

using a centrifuge

Invisorb® DNA Blood mini HTS 96 Kit/ C	4 x 96 preparations	1031300300
Invisorb® DNA Blood mini HTS 96 Kit/ C	24 x 96 preparations	1031300400

using a vacuum manifold

Invisorb® Blood mini HTS 96 Kit/ V	4 x 96 preparations	7031310300
Invisorb® Blood mini HTS 96 Kit/ V	24 x 96 preparations	7031310400

using a robotic station

Invisorb® Blood mini HTS 96 Kit/ R	4 x 96 preparations	7131300300
Invisorb® Blood mini HTS 96 Kit/ R	24 x 96 preparations	7131300400

using the X-tractor Gene™, Corbett Robotics

Invisorb® Blood mini HTS 96 Kit/ X	4 x 96 preparations	7131310300
Invisorb® Blood mini HTS 96 Kit/ X	24 x 96 preparations	7131310400

using the epMotion® 5075 VAC, Eppendorf

Invisorb® Blood mini HTS 96 Kit/ ep	4 x 96 preparations	7131320300
Invisorb® Blood mini HTS 96 Kit/ ep	24 x 96 preparations	7131320400