FastGene®

Blood & Tissue gDNA Extraction Kit

Catalog #: FG-70005
FG-70050
FG-70250

+49 2421 55496 0
+49 2421 55496 11
info@nippongenetics.eu
www.nippongenetics.eu

Table of contents

Kit contents

Cat. No.: FG-FG-70005 (5 preps)	
Lysis buffer (BTL)	1.2 ml
Tissue lysis buffer (T)	1 ml
Wash buffer 1 (BTW1)	2.45 ml
Wash buffer 2 (BTW2)	0.6 ml
Elution buffer (BTE)	1 ml
Proteinase K solution (20 mg/ml)	1.8 ml
FastGene® DNA binding column (with collection tubes)	5
2 ml collection tubes	5
User manual	1
Cat. No.: FG-70050 (50 preps)	
Lysis buffer (BTL)	12 ml
Tissue lysis buffer (T)	10 ml
Wash buffer 1 (BTW1)	22.4 ml
Wash buffer 2 (BTW2)	6 ml
Elution buffer (BTE)	10 ml
Proteinase K reconstitution buffer	1.8 ml
Proteinase K (lyophilized)	30 mg
FastGene® DNA binding column (with collection tubes)	50
2 ml collection tubes	50
User manual	1
Cat. No.: FG-70250 (250 preps)	
Lysis buffer (BTL)	55 ml
Tissue lysis buffer (T)	45 ml
Wash buffer 1 (BTW1)	112 ml
Wash buffer 2 (BTW2)	26 ml
Elution buffer (BTE)	50 ml
Proteinase K reconstitution buffer	6.5 ml
Proteinase K (lyophilized)	130 mg
FastGene® DNA binding column (with collection tubes)	250
2 ml collection tubes	250
User manual	1

IMPORTANT NOTICE:
Upon receipt of FastGene® Blood & Tissue gDNA Extraction kit store Proteinase K at -20°C!

Storage and stability

Store the FastGene® Blood & Tissue gDNA Extraction kit at room temperature (15-25 °C). Under these conditions FastGene® Blood & Tissue gDNA Extraction kit components are guaranteed for 18 month after manufacture. However, store included Proteinase K immediately upon receipt at -20°C. It is recommend to aliquot Proteinase K to avoid repeated freeze-and-thaw cycles. Storing Proteinase K at 4°C or room temperature will reduce performance.

Reagents and material to be supplied by the user

The following reagents have to be supplied by the user:

- 2-Propanol (≥99.5 %)
- 96-100 % ethanol
- Some samples may require phosphate-buffered saline (PBS)

The following consumables are required:

- Sterile Gloves
- Sterile, DNase -free pipet tips
- 1.5 ml reaction tubes, DNase-free

The following equipment is needed:

- Pipettes
- High Speed Centrifuge for 1.5 and 2 ml reaction tubes
- Homogenizer
- Vortex mixer
- Heated dry bath

Safety information

The following components of the kit contain hazardous contents. Wear gloves and goggles and follow the safety instructions given in this section.

GHS Classification (Hazard and precaution phrases)

Component of the kit*	Hazardous content	GHS Symbol	Hazard Phrases	Precaution Phrases
Buffer BTL Buffer BTW1	Guanidinium hydrochloride 30-50%	 DANGER	H302; H315; H319 H332	P280; P301+P312+P330; P302+P352+P313; P304+P340+P312; P305+P351+P338; P337+P313
Proteinase K	Proteinase K	 DANGER	H315; H319; H334	P261sh+P342+P311; P302+P352; P304+P340+P312

Hazard Phrases

H302 + H332	Harmful if swallowed.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H334	May cause allergy or asthma symptoms or breathing difficulties if inhaled.

Hazard Phrases

P301+P312+P330	If swallowed: Rinse out mouth. Call a POISON CENTER or doctor/physician if you feel unwell.
P302+P352+P313	If on skin: Wash with plenty of water and soap. If skin irritation persists: Get medical advice/attention
P304+P340+P312	If inhaled: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER or doctor/physician if you feel unwell.
P305+P351+P338	If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+P313	If eye irritation persists: Get medical advice/attention.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P261sh+P342+P311	Avoid inhalation of dust/vapor. In case of respiratory symptoms: Call a POISON CENTER or doctor/physician.

Description of FastGene® Blood & Tissue gDNA Extraction kit

Specification

The FastGene® Blood & Tissue gDNA Extraction kit is designed for isolation of genomic DNA from whole blood, serum, plasma and tissue. In addition, it can be used for genomic DNA extraction from cultured cells.

Sample volume	≤ 200µl whole blood (fresh or treated with anticoagulants) ≤ 25 mg tissue ≤ 5 x 10^6 cultured cells
Typical RNA yield	3-6 µg from 200 µl whole blood 15-35 µg from 22 mg liver mice tissue
Average operation time	25 min / prep (Blood) 20 min / prep (Tissue, excluding lysis)
Elution volume	100 µl

Principle

The FastGene® Blood & Tissue gDNA Extraction kit purifies genomic DNA from mammalian blood and tissues as well as cultured cells. The isolated DNA can be used for a variety of downstream applications e.g. PCR, qPCR, southern blot, enzymatic reactions and much more. For lysis the sample is treated with an Proteinase K-containing lysis buffer BTL. To achieve complete lysis of tissues, tissues are incubated with an additional buffer containing Proteinase and SDS for at least 1-3h before buffer BTL is added. Samples can be incubated overnight as well. Addition of ethanol provides appropriate DNA binding conditions to the silica membrane of the FastGene® DNA column.
In following steps contaminations are efficiently washed away with the supplied buffers BTW1 and BTW2 from the column. High-quality genomic DNA is subsequently eluted in 100 µl BTE buffer. The purified genomic DNA is ready for downstream applications or can be stored at - 20°C in a freezer.

Sample preparation

Genomic DNA can be isolated from fresh blood directly. Blood treated with anticoagulants like heparin, EDTA or citrate, can be used either directly or upon storage at room temperature, 4°C or frozen at -20°C or -80°C. For long-term storage it is recommended to store anticoagulated blood at -80°C to prevent DNA degradation.
Blood sample can be directly processed using the FastGene® Blood & Tissue gDNA Extraction kit. Some mammalian tissues might be difficult to lyse. For these samples it is recommended to incubate samples in Proteinase K and SDS-containing buffer T at 55°C over night. In addition some samples may be treated with a mechanical homogenizer, like the FastGene® Mixy Professional tissue grinder (NG010), prior to lysis. Grind the sample to a fine powder in the presence of liquid N_2. Take care that the sample does not thaw during or after grinding or weighing and add the frozen powder to an appropriate aliquot of lysis buffer BTL.
For downstream processes that require RNA-free genomic DNA it is recommended to perform RNase digestion after lysis: Add 20 µl RNase A (10mg/ml, not provided) to the sample and incubate for 5 min at room temperature (RT).
Make sure to not use a higher amount of starting material since this can result in decreased yield and purity of the eluted genomic DNA.

DNA elution

Usually, genomic DNA can be eluted by 100 µl buffer BTE. Please note, that it is recommended to heat the elution buffer to 70°C prior to all elution methods. Next to the standard elution method, yield and/or concentration of the eluate can be increased by the following procedures. A high yield of genomic DNA can be achieved by performing two subsequent elution steps using 100 µl buffer BTE each. A high concentration of genomic DNA can be achieved by performing one elution step using 60 µl elution buffer BTE. However, with this method total yield will be decreased. To achieve a high yield and high concentration of genomic DNA 100 µl of elution buffer BTE are applied, incubated for 3 min and centrifuged. Subsequently, the eluate is re-applied to the column, incubated for 3 min and centrifuged.
Elution can also be performed using Tris-EDTA buffer (TE) or nuclease-free water. Please make sure that elution buffer has a pH ≥ 8.

DNA quantification, quality and storage

We recommend to determine the quantity and quality of isolated genomic DNA to ensure best conditions for every downstream application. The easiest way to determine the concentration and purity of isolated DNA is to measure the absorbance at 260 nm and 280 nm with a spectrophotometer. Pure nucleic acids have an A260/A280 ratio of 2.0. On that account a ratio value of 1.8-2.0 represents 90-100% pure nucleic acid.
To ensure DNA stability keep genomic DNA frozen at -20°C.

Preparation of working solutions

First wash buffer BTW1

5 preps	50 preps	250 preps
Add 1.05 ml 2-propanol to 2.45 ml BTW1 and mix	Add 9.6 ml 2-propanol to 22.4 ml BTW1 and mix	Add 48 ml 2-propanol to 112 ml BTW1 and mix

Second wash buffer BTW2

5 preps	50 preps	250 preps
Add 2.4 ml ethanol* to 0.6 ml BTW2 and mix	Add 24 ml ethanol* to 6 ml BTW2 and mix	Add 104 ml ethanol* to 26 ml BTW2 and mix

*96-100% ethanol

Lyophilized Proteinase K (only 50 and 250 preps)

50 preps	250 preps
Add 1.5 ml Proteinase K reconstitution buffer to a vial of lyophilized Proteinase K	Add 1.5 ml Proteinase K reconstitution buffer to a vial of lyophilized Proteinase K. Reconstitute by mixing and transfer to bottle with Proteinase K Reconstitution buffer.

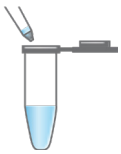
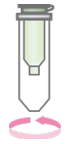
In order to collect the Proteinase K on the bottom of the vial spin down the powder by using a centrifuge before opening the tube. Add the indicated volume of the Proteinase K Reconstitution buffer, mix gently by tapping the tube. Do not vortex Proteinase K! Dissolved Proteinase K can be stored in aliquoted tubes at -20 ˚C. We do not recommend to refreeze and thaw the enzyme.

Genomic DNA extraction protocol

FastGene® Blood & Tissue gDNA Extraction quick protocol

Before starting the purification, please ensure that buffer BTW1, buffer BTW2 and Proteinase K are prepared accordingly (see chapter “Preparation of working solutions”).

All centrifugation steps are carried out at room temperature and at ≥ 10,000 x g.

Step	Blood protocol	Tissue protocol
1. Sample quantity	≤ 200µl whole blood ≤ 5 x 10^6 cultured cells	< 25 mg animal tissues
2. Lysis	 25 µl Proteinase K 200 µl buffer BTL 55°C 15-20 min	 25 µl Proteinase K 180 µl buffer T 55°C 1-3 h 200 µl buffer BTL 55°C 10 min
3. Optimize DNA binding conditions	 300 µl ethanol (96-100 %) Mix thoroughly	
4. DNA binding	 Load onto FastGene® DNA binding column Centrifuge 1 min	
5. Protein elimination	 500 µl of buffer BTW1 Centrifuge 1 min Transfer column in new 2.0 ml collection tube	
6. Desalination	 600 µl of buffer BTW2 Centrifuge 1 min	
7. Removal of BTW2	 Centrifuge at full speed for 2 min at RT Transfer spin column to new 1.5 ml collection tube	
8. Elution of DNA	 Add 100 µl of buffer BTE to membrane center Incubate 1 - 3 min at RT Centrifuge at full speed 1 min	

FastGene® Blood & Tissue gDNA Extraction detailed protocol

Before starting the purification, please ensure that buffer BTW1, buffer BTW2 and Proteinase K are prepared accordingly (see chapter "Preparation of working solutions"). Set a dry bath or water bath to 55°C. Preheat elution buffer BTE to 70°C.

1. Prepare samples in a tube (not provided in the kit). Proceed the next step as quickly as possible.

- ≤ 200µl whole blood (fresh or treated with anticoagulants)
  - If less than 200 µl are used, add PBS to a final volume of 200 µl
- ≤ 25 mg tissue
  - Cut tissue in small pieces
  - Difficult to lyse samples are recommended to be treated with a mechanical homogenizer. Grind the sample to a fine powder in the presence of liquid N2. Take care that the sample does not thaw during or after grinding or weighing.
- ≤ 5 x 10^6 cultured cells
  - Resuspend cell pellet in 200 µl PBS

For blood and cultured cells: Please proceed with Step 4, for tissues proceed with step 2.

2. Add 180 µl buffer T and 25 µl Proteinase K to the prepared tissue and vortex to mix

3. Incubate samples at 55°C for at least 1-3 h or until complete lysis is obtained.
   Some samples might require over night incubation for complete lysis.

4. *Optional: If RNA-free DNA is required add 20 µl RNase A (10 mg/ml) and incubate for 5 min at RT*

5. Add 200 µl lysis buffer BTL to the sample and vortex.
   a. Tissue: incubate for 10 min at 55°C
   If some particles remained insoluble, centrifuge for 5 min at ≥ 10,000 x g and transfer the supernatant to a new microcentrifuge tube (not provided). Proceed with using the supernatant only.
   b. Blood and cultured cells: Add 25 µl Proteinase K and incubate for 15 – 20 min at 55°C.
   Color of blood lysate should turn brownish. If not, vortex and increase incubation time to 30 min.

6. Add 300 µl ethanol (96-100 %) and mix by vortexing.

7. Take a FastGene® DNA binding column placed in a collection tube. Load up to 700 µl of the sample and centrifuge ≥10,000 x g for 1 min at room temperature (20-25°C).

8. Add 500 µl of buffer BTW1* and centrifuge at ≥ 10,000 x g for 1 min at room temperature (20-25°C), discard the flow-through and re-insert the spin column to a new 2 ml collection tube.
   **Make sure that 2-propanol is added to buffer BTW1 (see chapter "Preparation of working solutions").*

9. Add 600 µl of buffer BTW2* and centrifuge at ≥ 10,000 x g for 30 s at room temperature (20-25°C), discard the flow-through and re-insert the spin column in the 2 ml collection tube.
   **Make sure that ethanol is added to buffer BTW2 (see chapter "Preparation of working solutions").*

10. Centrifuge at full speed for 2 min at room temperature (20-25°C) to remove residual buffer BTW2. Transfer spin column to a new 1.5 ml collection tube.

11. Add 100 µl of buffer BTE to the center of the membrane in the FastGene® DNA binding column and incubate for 1 min at room temperature. Centrifuge at full speed for 1 min at room temperature (20-25°C) in order to elute the purified genomic DNA.
   **For optimal yields, it is recommended to heat elution buffer BTE to 70°C prior to elution. See section DNA Elution for optimized elution methods.*

Troubleshooting

Problem	Possible cause	Suggestions
No or low DNA yield and/or quality	Too small amount of starting material	Increase starting material up to the material specific recommended amount
	Immoderate amount of starting material	Reduce starting material to the material specific recommended amount
	Insufficient homogenization or disruption of starting material	Increase incubation time with the lysis buffer and vortex vigorously immediately after addition of lysis buffer. Make sure tissues are completely disrupted by using a mechanical homogenizer prior to lysis Avoid repeated freeze-and-thaw cycles of Proteinase K. Store reconstituted Proteinase K in aliquots at -20° for 12 months.
	Incomplete elution of DNA from spin column membrane	Repeat elution step with prior warming the elution buffer BTE to 70 °C
	Incorrect application of reagents	Be sure to comply with the instruction Prepare Proteinase K, buffer BTW1 and BTW2 accordingly Add ethanol to lysates before adding to the column
	Acidic buffer or water used for DNA dilution	As DEPC treated water becomes weakly acidic and decreases the absorbance value, please use elution buffer BTE, TE buffer etc.
	For cultured cells: Medium was not removed efficiently from cultured cells	Please completely remove the medium from the cell pellet. Residual medium leads to insufficient lysis procedure
Filter column is blocked	Immoderate amount of starting material	Reduction of starting material to the material specific recommended amount
	Too small amount of starting material	Increasing of starting material up to the recommended amount

Problem	Possible cause	Suggestions
Filter column is blocked (continued)	Insufficient homogenization or disruption of starting material	Complete homogenizing of starting material and increasing of centrifugation time Make sure, insoluble particles are centrifuged after lysis and proceed with supernatant only
DNA degradation	DNase contamination	Decontamination of all by user supplied plastics, reagents and work equipment
Suboptimal performance in downstream applications	RNA contamination	If RNA-free genomic DNA is required, RNase digestion must be performed prior to adding buffer BTL
	Salt contamination	Make sure to dry membrane by centrifugation prior to elution. In some cases it may be good to repeat washing step with the second wash buffer (BTW2). BTW2 must have room temperature.
	Incorrect storage of DNA	Keep diluted DNA on ice and store DNA for long term at - 20°C or colder.
	Residual ethanol	After washing with buffer BTW2, dry the membrane according to the protocol. When you remove the column, please make sure that the column is not in touch with the liquid inside the collection tube.. Add the elution buffer BTE to the center of the membrane. Carryover of Ethanol will affect downstream applications.

Ordering information

Catalog #	Product	Content
FG-70005	FastGene® Blood & Tissue gDNA Extraction Kit	5 preparations
FG-70050	FastGene® Blood & Tissue gDNA Extraction Kit	50 preparations
FG-70250	FastGene® Blood & Tissue gDNA Extraction Kit	250 preparations
NG010	Tissue Grinder Mixy Professional	1
NG006	Pestles for Tissue Grinder Mixy Professional	100

Contact information

Nippon Genetics Europe GmbH
Mariaweilerstraße 28a
D-52349 Dueren
Phone: +49 (0) 2421 55496-0
Email: info@nippongenetics.eu

For more detailed product information, contact details, questions, or trouble shooting please visit our website www.nippongenetics.eu

FastGene® is a registered trademark of Nippon Genetics Europe GmbH.