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INSTRUCTION

Kit for human DNA isolation from formalin fixed paraffin embedded tissue «DNA-Tissue-M» by TS 21.20.23-012-97638376-2019

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IVD

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Introduction

Target analyte. Kit **«DNA-Tissue-M»** is used at the pre-analytic stage. The obtained human genome DNA is not defined as a target analyte.

Scientific validity. Tissue's formalin fixation, posting according to a particular protocol and paraffin embedding for the subsequent producing of preparations is generally accepted method of preparing samples for histological examination. This approach allows to preserve tissue's structure and to store the material for a long time.

Archives that store tissue samples in paraffin blocks are essential material for large-scale retrospective research including ones using techniques not available at the time of archiving.

Today, as more and more information on the molecular basis of the pathogenesis of different diseases becomes available, DNA and RNA studies in histological material are necessary for scientific research and become a part of routine diagnostic practice.

There are different ways of dewaxing, most common of which is xylene treatment, which is normally carried out in a heating solution. Some additional measures could be taken to improve the quality of DNA or RNA preparation immediately before the start of extraction. This is heat treatment and enzyme processing using proteinase K. The use of elevated temperatures during incubation of the sample is justified by the fact that reversible damage to nucleic acids, caused by formalin, is repaired under existing conditions.

Nucleic acids isolated from tissue in paraffin block as required can be used for PCR and RT-PCR, sequence analysis, microarray analysis and for other molecular biological techniques.

Application area – clinical laboratory diagnostics.

Indications and contraindications

Indications: Kit «DNA-Tissue-M» is recommended to be used at the pre-analytic stage of clinical laboratory tests. The extracted human genome DNA from formalin fixed paraffin embedded tissue (FFPE-blocks) is suitable for PCR studies.

Contraindications: when used by specially trained personnel for use as intended there are no contraindications.

1. Intended use

Intended use: Kit «DNA-Tissue-M» is designed to extract human genome DNA from formalin fixed paraffin embedded tissue (FFPE-blocks) by the method based on sample lysis, nucleic acid binding on magnetic particle surfaces, several rounds of flushing and elution for subsequent clinical laboratory diagnostic tests by PCR.

Functionality: Kit «DNA-Tissue-M» is designed to provide pre-analytic stage of molecular genetic analysis.

Human genome DNA, extracted from formalin fixed paraffin embedded tissue (FFPE-blocks) can be used for subsequent clinical laboratory tests, in particular in oncology by PCR.

For the further research of DNA, extracted from the kit, the following medical devices may be applied:

- Kit for the detection of somatic mutations in the EGFR gene from the samples of formalin fixed paraffin embedded tissue (Test-EGFR-tissue) by TS 21.20.23-005-97638376-2016, produced by «TestGene» LLC (Registration certificate № FSSH 2018/7670 dated 04.10.2018);
- Kit for the detection of somatic mutations in the NRAS gene from the samples of formalin fixed paraffin embedded tissue (Test-NRAS-tissue) by TS 21.20.23-008-97638376-2016, produced by «TestGene» LLC (Registration certificate № FSSH 2018/7771 dated 02.11.2018);
- Kit for the detection of somatic mutations in the KRAS gene from the samples of formalin fixed paraffin embedded tissue (Test-KRAS-tissue) by TS 21.20.23-006-97638376-2016, produced by «TestGene» LLC (Registration certificate № FSSH 2018/7776 dated 02.11.2018);
- Kit for the detection of somatic mutations in the BRAF gene from the samples of formalin fixed paraffin embedded tissue (Test-BRAF-tissue) by TS 21.20.23-007-97638376-2017, produced by «TestGene» LLC (Registration certificate № FSSH 2019/9187 dated 07.11.2019).

Potential users of medical product

Kit is designed for professional use in medical institutions and diagnostic laboratories. The professional level of potential consumers is a doctor of clinical laboratory diagnostics, a medical laboratory technician.

2. Principle of the method

Type of tested sample. Material for extraction of nucleic acid is formalin fixed paraffin embedded tissue (FFPE-blocks).

Principle of the method. The basis of the method is based on dewaxing of FFPE-blocks (by incubation with xylene or mineral oil) and proteinase processing and subsequent rehydration with ethanol (95%).

After sample's lyse, nucleic acids in it bind to magnetic microbeads. Then they should be washed with wash buffers №1 and №2 from the kit. After several wash cycles the pellet of magnetic microbeads must be dried and nucleic acids can be eluted.

Kit's functionality allows it to be used for automated extraction for DNA.

Method limitations. Contamination of biological material is possible at the sample preparation stage or at the stage of DNA extraction.

Contamination can be detected by a negative control sample that must accompany the start of PCR.

Package integrity impaired during transportation.

Use of kit with expired shelf life.

Violation of the conditions of storage and transportation of samples.

Total time of DNA isolation procedure from 1 sample is more than 3 hours.

3. Kit contents

Kit «DNA-Tissue-M» in forms:

1) «DNA-Tissue-M-50» for 50 preparations;

2) «DNA-Tissue-M-100» for 100 preparations;

3) «DNA-Tissue-M-100» for 100 preparations with magnetic separation rack.

Kit contents

Table 1 – Contents of the kit «DNA-Tissue-M»

№ п п	Reagent	Description	Quantity		
			«DNA-Tissue-M-50» for 50 preparations	«DNA-Tissue-M-100» for 100 preparations;	«DNA-Tissue-M-100» for 100 preparations with magnetic separation rack
1	Lyse	Colorless clear liquid	1 vial, (10 ml)	1 vial, (20 ml)	1 vial, (20 ml)
2	DNA Binding buffer	Colorless clear liquid	1 vial, (15 ml)	1 vial, (30 ml)	1 vial, (30 ml)
3	Wash buffer №1	Colorless clear liquid	1 vial, (25 ml)	1 vial, (50 ml)	1 vial, (50 ml)
4	Wash buffer №2	Colorless clear liquid	1 vial, (15 ml)	2 vials, (15 ml each)	2 vials, (15 ml each)
5	Proteinase K Diluent	Colorless clear liquid	1 vial, (2,5 ml)	1 vial, (5 ml)	1 vial, (5 ml)
6	Proteinase K	White powder	1 vial, (50 mg)	1 vial, (100 mg)	1 vial, (100 мg)
7	Eluent	Transparent colorless liquid	1 vial, (5 ml)	1 vial, (10 ml)	1 vial, (10 ml)
8	Magnetic microbeads	Brown liquid	1 tube, (600µl)	1 tube, (1200µl)	1 tube, (1200µl)
9	Magnetic separation rack	Separation rack with volume of 1.5-2.0 ml with magnets	-	-	1 unit

Calibrators and control materials are not used in the kit.

The kit contains no remedies for medical use as well as substances of human or animal origin.

Sample does not contain any other ingredients that may influence the procedure.

4. Kit characteristics

4.1 Technical and functional characteristics

Table 2 – Technical and functional characteristics of the kit «DNA-Tissue-M»

Indicator name	Characteristics and standards
1. Technical characteristics	
1.1. Aspect	
1.1.1 «DNA-Tissue-M-50» for 50 preparations	
DNA Binding buffer	Colorless clear liquid
Lyse	Colorless clear liquid
Proteinase K Diluent	Colorless clear liquid
Proteinase K	White powder
Magnetic microbeads	Brown liquid
Wash buffer №1	Colorless clear liquid
Wash buffer №2	Colorless clear liquid
Eluent	Colorless clear liquid
1.1.2 «DNA-Tissue-M-100» for 100 preparations	
DNA Binding buffer	Colorless clear liquid
Lyse	Colorless clear liquid
Proteinase K Diluent	Colorless clear liquid
Proteinase K	White powder
Magnetic microbeads	Brown liquid
Wash buffer №1	Colorless clear liquid
Wash buffer №2	Colorless clear liquid
Eluent	Colorless clear liquid
1.1.3 «DNA-Tissue-M-100» for 100 preparations with magnetic separation rack.	
DNA Binding buffer	Colorless clear liquid
Lyse	Colorless clear liquid
Proteinase K Diluent	Colorless clear liquid
Proteinase K	White powder
Magnetic microbeads	Brown liquid

Wash buffer №1	Colorless clear liquid
Wash buffer №2	Colorless clear liquid
Eluent	Colorless clear liquid
Magnetic separation rack	Separation rack with volume of 1.5-2.0 ml with magnets
1.2 Physical and chemical indicators	
Hydrogen ion concentration, pH	
DNA Binding buffer	min 6.0 pH, max 8.0 pH
Wash buffer №1	min 6.0 pH, max 8.0 pH
Wash buffer №2	min 6.0 pH, max 8.0 pH
1.3. Compound	According to point 1.4 by TS 21.20.23-012-97638376-2019
1.4. Labelling	According to point 4 by TS 21.20.23-012-97638376-2019
1.5. Package	According to point 5 by TS 21.20.23-012-97638376-2019
2. Functional characteristics	
2.1 Purity of extracted DNA, A260/280	1.7
2.2. Concentration of the extracted DNA from 30 mg of formalin fixed paraffin embedded tissue, ng/μl	5
2.3. No contamination	Negative result with a negative control sample in the control PCR

Note: when performing the control PCR, deionized sterile water free of DNA/RNA shall be used as a negative control sample.

DNA was extracted from different types of formalin fixed paraffin embedded human tissues (liver, kidney, spleen, colon, brain, muscles, lung) for appraisal of the kit's «DNA-Tissue-M» functionality according to the kit's instruction.

The quantity of formalin fixed paraffin embedded tissue is 30 mg.

In each section from the FFPE block, the area of the fixed tissue fragment was up to 250 mm^2, the thickness of the cut is up to 10 μm.

After the genomic DNA extraction, DNA purity (expressed in terms of the ratio of absorbance at 260/280 nm) and DNA concentration (expressed in μl)/ml) were evaluated.

The results of the study are presented in table 3.

On the basis of the obtained results of DNA extraction by the kit «DNA-Tissue-M» from formalin fixed paraffin embedded human tissues (liver, kidney, spleen, colon, brain, muscles, lung) it is possible to conclude the following:

1. Concentration of the extracted DNA from 30 mg formalin fixed paraffin embedded tissue is at least 5 ng/μl, which is sufficient for further PCR reaction.

2. The purity of DNA extraction is at least 1.7, which is acceptable for further PCR reaction.

Table 3 – Results of the study of functional characteristics of «DNA-Tissue-M».

Tissue type	Quantity of formalin fixed paraffin embedded tissue, mg	Concentration of the extracted DNA, ng/μl	DNA purity, ratio of absorbance at 260/280 nm
liver	30	15.9	1.7
kidney	30	15.6	1.8
spleen	30	18.0	1.8
colon	30	16.6	1.7
brain	30	17.7	1.7
muscles	30	6.2	1.7
lung	30	17.9	1.8

4.2 Clinical efficacy

On the basis of the results of clinical trials, the effectiveness of the medical product when used as intended by PCR clinical and laboratory tests with the samples of human genome DNA from 110 samples of formalin fixed paraffin embedded human tissue (colon, lung, skin) using registered medical devices for in vitro diagnostics has been confirmed.

To assess inter-series convergence, a DNA extraction from the samples of formalin fixed paraffin embedded tissue (FFPE-blocks) was

conducted in two series.

Thus, the quality, safety and effectiveness of the medical device was checked in 220 tests.

100 % result of the study of medical device's effectiveness when used as intended in a series of 220 experiments confirmed the clinical effectiveness and with confidence probability of 95% it is 100% (95% confidence interval:96%-100%).

ща interval

5. Possible problems and its suggestions

The border risk zone includes hazards:

- loss of functional properties of reagents due to inadequate transport, storage or operation;
- use of cut-off from FFPE-block in quantities insufficient for DNA extraction;
- cross-contamination of samples when preparing the cuts-off from FFPE-block;
- failure to meet sampling, analysis and utilization due to the work of unskilled personnel;
- use of an unsuitable kit (use after expiry or in case of packaging violation).

No risks have been identified in the area of the unacceptable zone.

The cumulative residual risk of using a medical device «Kit for human DNA isolation from formalin fixed paraffin embedded tissue «DNA-Tissue-M» by TS 21.20.23-012-97638376-2019» manufactured by «TestGene» LLC is acceptable, the benefits of its use exceed the risk.

6. Safety precautions

Class depending on the potential risk of use is 2a in accordance with the nomenclature classification of medical devices approved by order of the Ministry of Health of the Russian Federation of 06.06.2012 N 4n.

The work with clinical material when using the kit «DNA-Tissue-M» should be carried out according to methodological recommendations "Obtainment, transportation, storage of clinical material for PCR diagnostics», Federal Budget Institution of Science "Central Research Institute of Epidemiology" Moscow, 2012.

The hazardous components of the kit according to «the Globally Harmonized System of Classification and Labelling of Chemicals (GHS)» are shown in table 4.

Table 4 – Hazardous components of the kit

Reagent	Hazardous components	Hazard pictograms	Risk statements	Precautionary statements
DNA Binding buffer	Guanidine isothiocyanate CAS 593-84-0		302, 315, 319	264, 280, 301+312, 302+352, 305+351+338, 330, 332+313, 337+313
Wash buffer №1	Guanidine isothiocyanate CAS 593-84-0		302, 315, 319	264, 280, 301+312, 302+352, 305+351+338, 330, 332+313, 337+313
Proteinase K	Proteinase K CAS 39450-01-6		332, 334	261, 272, 280, 302+352, 304+340, 333+313, 342+311, 363

Risk Statements

H 302	Harmful if swallowed
H 315	Causes skin irritation
H 319	Causes serious eye irritation
H 332	Harmful if inhaled
H 334	May cause allergy or asthma symptoms or breathing difficulties if inhaled

Precautionary statements

P 261	Avoid breathing vapours
P 264	Wash your hand thoroughly after handling
P 272	Contaminated work clothing should not be allowed out of the workplace.
P 280	Wear protective gloves/protective clothing/eye protection/face protection
P 301+312	IF SWALLOWED: Call a POISON CENTER/doctor/…/if you feel unwell.
P 302+352	IF ON SKIN: Wash with plenty of water
P 304+340	IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P 305+351+338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do – continue rinsing.
P 330	Rinse mouth.
P 332+313	If skin irritation occurs: Get medical advice/attention.
P 333+313	If skin irritation or a rash occurs: Get medical advice/attention.
P 337+313	If eye irritation persists get medical advice/attention.
P 342+311	If experiencing respiratory symptoms: Call a POISON CENTER/doctor/…
P 363	Wash contaminated clothing before reuse.

The symbol on the label indicates additional safety information in the Instructions.

Aspiration hazard symbol

When using reagents of the kit «DNA-Tissue-M» there is no environmental pollution.

The work shall be carried out in the laboratory for biomolecular (PCR) testing of clinical material in compliance with the sanitary and epidemiologic rules SP 1.3.2322-08 «Safety work with microorganisms

belonging to III-IV pathogenicity groups (dangers) and parasitic diseases agents», SanPiN 2.1.7.2790-10 «Sanitary and Epidemiologic Requirements to the address with Medical Waste Products» and Methodical Instruction MU 1.3.2569-09 «Organization of the working process in laboratories using nucleic acid amplification methods in working with material, containing microorganisms of I–IV pathogenic groups».

The following requirements should always be fulfilled during the work:

1. Consider test samples as dangerous infections, organize work and storage in accordance with SP 1.3.2322-08 «Safety work with microorganisms belonging to III-IV pathogenicity groups (dangers) and parasitic diseases agents».

2. Remove and sterilize spilled samples and reagents using sanitizers in accordance with SP 1.3.2322-08 «Safety work with microorganisms belonging to III-IV pathogenicity groups (dangers) and parasitic diseases agents».

3. Laboratory process must be unidirectional. Testing is performed in separate rooms (areas). Start work in Isolation area, continue in Amplification and Detection area. Do not return samples, equipment and reagents in previous area.

4. Remove unused reagents, reagents with expired shelf life and utilized reagents in accordance with SanPiN 2.1.7.2790-10 «Sanitary and Epidemiologic Requirements to the address with Medical Waste Products».

5. Use and change disposable tips for automatic dispensers with filter. Utilize disposal plastic tableware at special container with sanitizer that can be used for medical waste disinfection.

6. Uviolize table surfaces, rooms for PCR during 30 minutes before and after work completion.

7. Use kit by designation according to this instruction.

8. Admit only specially trained staff to the work with the kit.

9. Do not use kit after the expiry date.

10. Do not use kit if the inner packaging is broken or if the external appearance does not correspond to the description.

11. Use disposal gloves, lab coats, protect your eyes while working with the samples and reagents. Wash hands thoroughly at the end of the work.

12. All components of the kit are non-toxic to humans in the concentrations used. If on skin or on mucous membranes, wash with plenty of water.

13. There are no necessary measures recommended for safety regarding to the influence of magnetic fields, external electrical effects, electrostatic discharges, pressure or differential pressure, overload, thermal ignition source.

In kit composition there are no materials of human or animal origin with potential infectious nature. So special safety precautions are not applicable during product using.

ATTENTION! Disinfection of biomaterial and reagents should be carried out by placing disposable plastic dishes (test tubes, tips), aspirators with trap flask for 20-24 hours in special containers with disinfection solution.

7. Equipment and materials

Equipment:

1. Sterile laminar box (e.g., BAVp-01-Laminar-S-1,2, Laminar Systems, Russia),
2. Thermostat for Eppendorf tubes from 25 to 100 °C (e.g., TERMO 24-15, Biokom, Russia),
3. Microcentrifuge for Eppendorf tubes to 14000 g (e.g., «MiniSpin», «Eppendorf», Germany),
4. Vortex (e.g., TETA-2, Biocom, Russia),
5. Separate set of automatic variable-volume pipettes (e.g., «Eppendorf», Germany),
6. Refrigerator from 2 °C to 8 °C,
7. Freezer from – 2 °C to – 40 °C.

Additionally, can be used:

8. Aspirators with trap flask (e.g., «FTA-1», Biosan, Latvia).

Materials and reagents not included in the kit:

1. Xylene or mineral oil (e.g., «Panreac» or similar),
2. Absolute ethanol (95%),
3. Disposal polypropylene microtubes for 1,5 ml, screw-capped or closed tightly, DNA and DNAse-free (e.g., Axygen, USA),
4. Racks for microtubes for 1,5 ml (e.g., InterLabService, Russia) and tips (e.g., Axygen, USA),
5. Magnetic separation rack with volume of 1.5-2.0 ml (e.g., InterLabService),
6. Disposal tips for variable volume pipettes with aerosol barrier for up to 100 µl and 1000 µl, DNA-free and DNAse-free (e.g., Axygen, USA),
7. Disposal tips for variable volume pipettes for up to 100 µl and 1000 µl, DNA-free and DNAse-free (e.g., Axygen, USA),
8. Disposal or individual coats and disposal gloves,
9. Containers with disinfection solution.

No other materials or reagents are required. No measuring equipment is required when using the kit.

8. Test samples

Material for extraction of nucleic acid is formalin fixed paraffin embedded tissue (FFPE-blocks).

Use a microtome to make cuts. In each cut from FFPE-block, the area of a fixed tissue shall be up to 250 mm2, ehe thickness of the cue shall be up to 10 µm.

For DNA extraction use 2 cuts from FFPE-block.

Before starting work consult the methodological recommendations "Obtainment, transportation, storage of clinical material for PCR diagnostics», Federal Budget Institution of Science "Central Research Institute of Epidemiology" Moscow, 2012.

8.1 Obtaining of biological material

Requirements for the preparation of tissue samples in FFPE-blocks:

8.1.1 When fixing tissue with formalin use 10% neutral formalin (pH from 7.0 to 7.6).

8.1.2 Use a fixation time of no longer than 24 hours.

8.1.3 Use only EDTA based reagents for decalcination.

ATTENTION! Samples after decalcination with formic or nitric acid are not suitable for molecular research.

8.1.4 Work in gloves, inside sweat or laminar cupboards, use disposable tools and consumables.

8.1.5 Avoid contact of fragments of biological material with each other and with any other biological material.

8.1.6 Total shelf life of formalin fixed paraffin embedded tissue (FFPE-blocks) not exceeding 3 years at temperatures between 15 and 25 C.

8.2 Interfering substances and method limitations

The influence of potentially interfering substances on the operation of the kit «DNA-Tissue-M» has been tested for substances encountered in DNA extraction and sample preparation procedures.

The following substances encountered in DNA extraction from formalin fixed paraffin embedded tissue (FFPE-blocks) are classified as PCR inhibitors: sodium dodecyl sulfate (included in DNA Binding buffer), ethanol (included in Wash buffer №1 and Wash buffer №2) and xylene (used for dewaxing of FFPE-blocks), which may be present in the eluate with DNA as a result of incomplete removal during the DNA extraction.

The maximum concentrations of interfering substance at which there is no influence on the amplification of the control sample are: sodium dodecyl sulfate - 0,007 µl/ml of DNA sample, ethanol – 5 µl/ml of DNA sample, xylene - 0,001 µl/ml of DNA sample.

The following substances encountered in sample preparation procedure are classified as PCR inhibitors:

- paraffin used to make FFPE-blocks,
- blood haemoglobin in the tissue sample,
- elements of histolysis and inflammation.

Tested concentrations of interfering substances that will occur during the normal use of the kit "DNA-Tissue-M" are shown in Table 5.

Table 5 – Potentially interfering substances that will occur in sample preparation procedure and their concentrations

Interfering substances	Concentration
Paraffin	0,001 μl/ml of DNA sample
Haemoglobin	0,35 μl/ml of DNA sample
Elements of histolysis and inflammation	necrosis at level >15 %

Based on the results of the study, these substances are removed during the DNA extraction using the kit «DNA-Tissue-M» and do not have an interfering effects.

8.3 Limitations on the use of the analyzed material

1. The analyzed material is not subject to use in case of violation of storage and transportation (conditions temperature, duration, multiple freezing-thawing).

2. Samples contaminated with foreign biological material are not permitted.

3. When preparing cuts from paraffin blocks, the risk of cross-contamination should be minimized by:

• the work in disposable powder-free gloves;

• the conduction the procedure in the PCR box or in the sterile laminar box;

• the use of disposable microtome blades and sterile tweezers;

• the utilization of the first two cuts from each block первые два среза с каждого блока утилизировать, the use of cuts from the third for molecular research;

• no use of water bath.

8.4 Criteria for the suitability of histological preparations for DNA extraction for further molecular genetic analysis of tumor cells

• According to the results of morphological examination, tumor complexes should occupy at least 60 % of the tissue area in the cut from FFPE-block.

• According to the results of morphological examination, areas of necrosis and haemorrhage taken together shall not exceed 15 % of the tissue area in the cut from FFPE-block.

If the sample does not meet at least one of the listed criteria, it is recommended to use another sample.

8.5 Storage conditions of test samples

Storage conditions of formalin fixed paraffin embedded tissue (FFPE-blocks):

- at a temperature from +15 to +25°C - no more than 3 years.

Storage conditions of extracted DNA:

- **AT A TEMPERATURE OF +4 °C – LESS THAN 24 HOURS,**
- **AT A TEMPERATURE FROM -18 TO -22 °C – LESS THAN A MONTH,**
- **AT A TEMPERATURE OF -70 °C – FOR A LONG STORAGE.**

9. Prepatation of the components for testing

Rigging, assembling, setting up, calibration of medical device is not required.

Crystal drop-out does not affect the quality of the kit. When crystallizing any solution, heat up the vial at 50°C and thoroughly mixed until the crystals are completely dissolved and the solution is homogenized.

Before starting the procedure, prepare the **solution of Proteinase K**.

Transfer the whole volume of «Proteinase K solution» into a bottle with dry «Proteinase K» and completely dissolve the "Proteinase K".

Before starting the procedure, prepare the **Wash buffer №1** and **Wash buffer №2.**

For «DNA-Tissue-M-50»:

1) add 12,5 ml ethanol (95%) to the «Wash buffer №1».

2) add 60 ml ethanol (95%) to the «Wash buffer №2».

Tick the check box on the bottle label to indicate that the operation is performed.

For «DNA-Tissue-M-100» and «DNA-Tissue-M-100» with magnetic separation rack:

1) add 25 ml ethanol (95%) to the «Wash buffer №1».

2) add 60 ml ethanol (95%) to each vial of «Wash buffer №2».

Tick the check box on the bottle label to indicate that the operation is performed.

!!! All components of the set must be carefully mixed before starting work.

10. Testing procedure

Admit only specially trained staff to the work.

Samples should be cut into sections immediately before extraction. In each section from the FFPE block, the area of the fixed tissue fragment shall be up to 250 mm^2, the thickness of the cut is up to 10 μm.

To obtain cuts, use a microtome.

To extract DNA, it is recommended to use 2 cuts from the FFPE-block.

The DNA extraction procedure is immediately preceded by dewaxing with xylene or mineral oil.

Dewaxing with xylene:

1. Place 2 cuts from FFPE-block in a microcentrifuge tube 1,5-2,0 ml.

2. Add 1200 μl xylene. Close the lid and incubate at room temperature until the paraffin is completely dissolved (usually for 2 min.) and vortex for 10 s.

3. Centrifuge for 2 min. at 11 000 g at room temperature (15-25 °C).

4. Remove the supernatant by pipetting without affecting the sediment.

5. Add 1200 µl ethanol (95%) to the pellet. Mix by vortexing (5 s.). The ethanol extracts residual xylene from the sample.

6. Centrifuge for 2 min. at 11 000 g at room temperature (15-25 °C).

7. Remove the supernatant by pipetting without affecting the sediment.

8. Open the tube and incubate at 60 °C for 10 min. or until all residual ethanol has evaporated. Residual ethanol can cause a little DNA.

Dewaxing with mineral oil:

1. Place 2 cuts from FFPE-block in a microcentrifuge tube 1,5-2,0 ml.

2. Add 1000 µl of mineral oil, mix by vortexing and incubate in thermostat for 10 min. at 60 °C, intermittently mixing solution in vortex.

3. Centrifuge for 2 min. at 11 000 g at room temperature (15-25 °C).

4. Remove the oil by pipetting without affecting the sediment.

5. Add 1000 µl of ethanol (95%). Close the lid and mix by vortexing for 10 s.

6. Centrifuge for 2 min. at 11 000 g at room temperature (15-25 °C). Remove the ethanol by pipetting without affecting the sediment.

7. Repeat paragraphs 4 and 5.

8. Incubate the tube in thermostat with an open lid for 10 min. at 60 °C. or until all residual ethanol has evaporated.

DNA extraction from the sample after dewaxing:

1. Resuspend the pellet in 180 µl of lyse, than add 25 µl of Proteinase K. Mix by vortexing for 5 s. without foaming. The tissue should be completely immersed in the lysing solution.

2. Incubate the mixture at 60 °C for 1-3 hours until lysis of tissue sample. During the incubation mix the solution by voxering for 5 s. or use thermomixer.

3. Mix by voxering for 5 s. and incubate for an hour at 90 °C. This stage is necessary to reverse formaldehyde modification of DNA.

Longer incubation times or higher incubation temperatures may result in more fragmented DNA.

4. Prepare in a separate tube a buffer with magnetic microbeads (mix 250 μl DNA buffer and 10 μl of buffer with well-mixed magnetic microbeads.

5. Cool the test tube with the lysed tissue sample at room temperature, remove drops from the inside of the lid by brief centrifugation, carefully mix the solution by pipetting up and down and transfer it to the tube with a prepared solution of magnetic microbeads. Mix by vortexing for 3-5 s.

6. Incubate the tube at room temperature for 5 min., mixing the solution by voxering 2-3 times for 1-2 s. or flipping the tube 2-3 times.

7. Remove drops by brief centrifugation and place the tube to the magnetic separation rack, wait until the microbeads gather on the tube walls (usually it takes 10 min) and discard the supernatant.

8. Transfer to the tube 700 μl of well-mixed wash buffer №1, completely resuspend magnetic microbeads by pipetting up and down or by vortexing.

9. Remove drops by brief centrifugation and place the tube to the magnetic separation rack, wait until the microbeads gather on the tube walls (usually it takes 1-2 min) and discard the supernatant.

10. Transfer to the tube 700 μl of well-mixed wash buffer №2, mix carefully by pipetting up and down or by vortexing.

11. Remove drops by brief centrifugation and place the tube to the magnetic separation rack, wait until the microbeads gather on the tube walls (usually it takes 1-2 min) and discard the supernatant.

12. Transfer to the tube 700 μl of well-mixed wash buffer №2, mix carefully by pipetting up and down or by vortexing.

13. Remove drops by brief centrifugation and place the tube to the magnetic separation rack, wait until the microbeads gather on the tube walls (usually it takes 1-2 min) and discard the supernatant.

14. Put the tube with an open lid in thermostat and incubate at 60 ºC for 8-10 min. to dry and remove residual ethanol.

15. Transfer to the tube 60-100 μl of buffer for elution. Resuspend carefully by pipetting up and down.

16. Incubate the tube with a closed lid in the thermostat at 60 °C for 10 min., while incubation mix carefully 2-3 times.

17. Place the tube to the magnetic separation rack, wait until the microbeads gather on the tube walls.

18. Transfer the supernatant, having extracted DNA. The sample is ready for PCR.

When producing PCR, it is recommended to keep a test tube with extracted DNA on the magnetic separation rack.

11. Possible problems and its suggestions

1. Little or no DNA in the eluate, reason and possible solution:

▪ insufficient sample lysis.

Proteinase K was stored at high temperatures for a prolonged time. Repeat the procedure using new samples and fresh Proteinase K. Make sure that the samles were thoroughly dehydrated. Residual formalin can inhibit the Proteinase K digest.

▪ low-percentage ethanol used instead of 95% ethanol. Repeat the purification procedure with new samples using 95% ethanol. Do not use denatured alcohol, which contains other substances, such as methanol or methylethylketone.

▪ Wash buffer №1 and wash buffer №2 prepared incorrectly. Make sure that wash buffer №1 and wash buffer №2 concentrates were diluted with the correct volume of 95% ethanol, as described in paragraph 9.

When crystallizing any solution it is necessary to warm up the vial with solution at 50 °C and thoroughly mix until the crystals are completely dissolved and the solution is homogenized.

2. Poor DNA in subsequent enzymatic reactions

▪ DNA is fragmented because of modification of formaldehyde.

Although incubation at 90°C removes most of the formaldehyde modifications, DNA extracted from FFPE-blocks may not work in enzymatic reactions as well as DNA extracted from fresh or frozen tissue. Short amplicons are recommended for PCP, <500 nucleotides.

▪ Reduced sensitivity. Determine the maximum volume of eluate suitable for your amplification reaction. Adjust the volume of eluate added to the amplification reaction accordingly. The elution volume can be adjusted proportionally.

▪ Wash buffers not mixed well. Salt and ethanol components of wash buffers №1 and №2 may have separated out after being unused for a long period. Always mix buffers thoroughly before each purification procedure.

▪ Incomplete drying of pellet from residual ethanol.

After complete supernatant removal, it is necessary to open the lid and incubate at 60 °C for 10 min. or until all residual ethanol has evaporated.

Residual ethanol can cause a little DNA.

If you have any questions, please contact support service of TestGene – see Section 14.

12. Storage, transportation and usage conditions

Storage.

Kit «DNA-Tissue-M» in the manufacturer's packaging must be stored in the supplier's warehouses in dry, ventilated areas.

Kit must be stored at room temperature 15-25°C and at a relative humidity of up to 90%. The atmosphere pressure is not controlled; it does not affect the quality of the product.

Proteinase K must be stored at temperature not above -18 °C.

Kit stored with violations are not to be used.

Transportation.

Kit «DNA-Tissue-M» must be transported in covered vehicles of all types in accordance with transport regulations.

Kit must be transported at temperature from +2°C to +30°C and at a relative humidity of up to 90%. The atmosphere pressure is not controlled; it does not affect the quality of the product.

Kits transported with violations of temperature conditions are not to be used.

Shelf life – 12 months from the date of acceptance of the manufacturer's QAD, if all conditions of transport, storage and operation are met.

Shelf life. 12 months. Kit with expired shelf life is not to be used.

Shelf life of opened components – 12 months when stored at room temperature of 15-25°C.

After opening the bottles and adding ethanol (95%) to Wash Buffer №1 and №2 shelf life is 6 months.

Store Proteinase K Diluent after dilution not more than 6 months at temperature below minus 18 °C.

Kit stored in violation of the regulated regime is not subject to use.

13. Utilisation

In accordance with Medical Waste class of the kits is A (epidemiologically safe waste with an approximate composition to solid household waste).

Kits that have become unusable because of shelf life expiration, are subjected to utilization in accordance with the requirements of SanPiN 2.1.7.2790-10 «Sanitary and epidemiologic requirements to the address with medical waste», reagents are assembled in a single-use labeled package of any colour (except yellow and red).

The remaining test tubes and materials are disposed in accordance with the guidelines for disinfection, pre-sterilization cleaning and sterilization of medical devices.

Liquid components are eliminated by draining into the sewage system with a preliminary watering of the reagent with tap water 1: 100 and removal of the packages rest as industrial or household garbage.

Consumer package of «DNA-Tissue-M» kit is subjected to mechanical destruction with removal of residues as industrial or household garbage.

Personnel carrying out the destruction of the kit must comply with the safety rules for carrying out a particular method of destruction.

14. Warranty oobligations, contact information

The manufacturer guarantees stable operation of the kit «DNA-Tissue-M» in compliance with storage conditions during expiry date.

If there are any complaints regarding the quality, send the information to the address:

Limited liability company «TestGene»
(TestGene LLC),
9, 44th Inzhenerny proyezd, Ulyanovsk, 432072, Russia
Tel.: +7 499 705-03-75
www.testgen.ru

Technical support service:
Tel.: +7 927 981 58 81
E-mail: help@testgen.ru

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CMC MEDICAL DEVICES & DRUGS S.L.
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