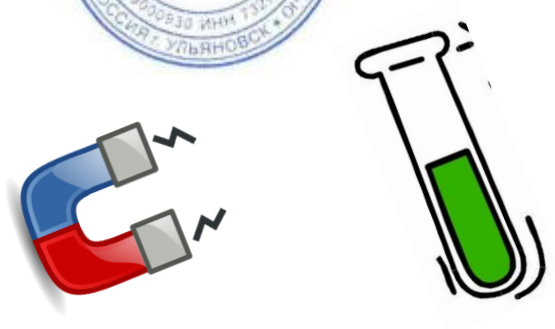
APPROVED BY
CEO of TestGene, LLC
Andrey N. Toropovsky
29 April 2019



INSTRUCTION FOR USE

Reagent Kit for Circulating Free DNA Extraction from Blood Plasma (DNA-Plasma-M-RT)

according to Technological Specification (TS) 21.20.23-010-97638376-2017

TestGene, LLC
9, 44th Inzhenerny proyezd, office 13, Ulyanovsk, 432072, Russia

CE IVD

Version 2 – revised on 29.04.2019

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Introduction

Target analyte. DNA-Plasma-M-RT reagent kit is used during the phase of preparation of a sample for the subsequent testing. The kit is not designed for isolation of human DNA as a target analyte.

Scientific validity.

DNA extraction is an important step in sample preparation. Many methods, such as amplification, reverse transcription, detection of accumulation of amplification products by real-time PCR, etc., cannot be performed directly on biological samples without preliminary purification of nucleic acids.[1]

DNA extraction is required for performing genetic tests used for scientific and medical purposes. In medical practice, DNA extraction is used for diagnosis of hereditary diseases, identification of risks of developing various hereditary diseases.

Circulating free DNAs are double stranded low-molecular-weight genomic DNA molecules that is fragmented into short (70-200 base pairs) and long (up to 21,000 base pairs) segments that are resistant to RNases and proteinases, but cleavable using DNase. There are two possible sources of cfDNA in the bloodstream: passive DNA release by apoptotic cells and necrotic cells and active DNA release by cell secretion[2].

Circulating free nucleic acids (cfNA) in plasma and in blood serum, obtained after extraction are an extremely valuable object of molecular-genetic analysis.

The use area of the reagent kit is clinical laboratory diagnostics, oncology, and prenatal diagnostics.

Indications and Contraindications for Use.

Indications for use: DNA-Plasma-M-RT reagent kit is recommended for extraction of circulating free DNA (cfDNA) from human peripheral venous blood plasma for subsequent use when performing tests during clinical laboratory diagnostics by allele-specific real time PCR.

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[1] O. S. Antonova, N. A. Korneva, Y. V. Belov, V. E. Kurochkin, Effective Methods of Nucleic Acid Isolation for Analysis in Molecular Biology. Review. // Scientific Instrument Engineering. - 2010. - Volume 20, No. 1. - P. 3-9.

[2] S. N. Tamkovich, V. V. Vlasov, P. P. Laktionov. Circulating Blood DNA and Their Use in Medical Diagnostics / S. N. Tamkovich, V. V. Vlasov, P. P. Laktionov // Molecular Biology. - 2008. - Vol. 42, No. 1. - P. 12-23.

Contraindications for Use: No.

1. Intended Use

Intended use: DNA-Plasma-M-RT reagent kit is designed for circulating free DNA (cfDNA) extraction from human peripheral venous blood plasma using the method based on the reversible binding of nucleic acids on the surface of magnetic beads, for subsequent analysis in clinical laboratory diagnostics by allele-specific real time PCR.

Functional purpose: DNA-Plasma-M-RT reagent kit is designed for use during preliminary analytical stage and cfDNA extracted from human blood plasma is not the basis for a diagnosis, but it can be used for subsequent testing in clinical laboratory diagnostics, in particular in the field of prenatal diagnostics and oncology when testing by allele-specific real time PCR.

For example, the following medical devices can be used together for subsequent allele-specific PCR:

- Reagent Kit for Mutation Detection of EGFR Gene Based on Real-Time PCR (Test-EGFR) in accordance with TS 9398-004-97638376-2015 designed as: 1) “Test-EGFR-12” for 12 extractions, 2) “Test-EGFR-24” for 24 extractions, Produced by TestGene, LLC (Registration Certificate No. RZN 2017/6267 of 19 September 2017,

- Diagnostic kits for identifying fetal DNA in maternal blood in accordance with TS У 9398-001-97638376-2012, produced by TestGene, LLC (Registration Certificate No. RZN 2015/2703 of 03 February 2016) and similar medical devices.

Potential users of the medical device

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

2. Method Principle

Type of sample to test

The human peripheral venous blood plasma is material for circulating free DNA extraction.

Detection Principle

The kit is based on the principle of reversible binding between DNA and the surface of magnetic beads. After lysis of the sample, the nucleic acids contained in it bind to magnetic beads. Then magnetic beads should be washed with wash buffers No. 1 and No. 2, which are part of the kit. After several washing cycles, the magnetic bead sediment must be dried, and then nucleic acids can be eluted.

Total time for DNA extraction from one sample is 70 minutes.

3. Reagent Kit Components

Design versions

DNA-Plasma-M-RT reagent kit is produced in three design versions:

1) DNA-Plasma-M-RT-25 for 25 extractions,

2) DNA-Plasma-M-RT-50 for 50 extractions,

3) DNA-Plasma-M-RT-50 for 50 extractions with a Magnetic separation rack.

DNA-Plasma-M-RT reagent kit is designed for extraction from 2 ml plasma.

Kit Components

Table 1 – Components of DNA-Plasma-M-RT reagent kit

No.	Component	Description	Design		
			DNA-Plasma-M-RT-25 for 25 extractions	DNA-Plasma-M-RT-50 for 50 extractions	DNA-Plasma-M-RT-50 for 50 extractions with Magnetic separation rack
1	DNA binding buffer	Transparent colorless liquid	1 bottle (90 ml)	2 bottles (90 ml each)	2 bottles (90 ml each)
2	Lysis buffer	Transparent colorless liquid	1 bottle (20 ml)	1 bottle (40 ml)	1 bottle (40 ml)
3	Magnetic Beads, MB	Brown suspension	1 tube (600 µl)	1 tube (1200 µl)	1 tube (1200 µl)

No.	Component	Description	Design		
			DNA-Plasma-M-RT-25 for 25 extractions	DNA-Plasma-M-RT-50 for 50 extractions	DNA-Plasma-M-RT-50 for 50 extractions with Magnetic separation rack
4	Wash buffer No. 1	Transparent colorless liquid	1 bottle (16 ml)	1 bottle (30 ml)	1 bottle (30 ml)
5	Wash buffer No. 2	Transparent colorless liquid	1 bottle (8 ml)	1 bottle (15 ml)	1 bottle (15 ml)
6	Eluent	Transparent colorless liquid	1 bottle (3 ml)	1 bottle (6 ml)	1 bottle (6 ml)
7	Magnetic separation rack	Holder for 1.5 ml and 15 ml tubes with 100*15*15 mm magnet	-	-	1 pc

The extraction kit does not include calibrators or control materials.

The kit does not contain medicinal products for medical use, substances of human or animal origin.

Note: the product does not contain other ingredients that may affect the procedure.

4. Reagent Kit Characteristics
4.1 Technical and Functional Characteristics

Table 2 – Technical and Functional Characteristics of DNA-Plasma-M-RT reagent kit.

Parameter Name	Characteristics and Standards
1. Technical Characteristics	
1.1. Visual appearance	
1.1.1 DNA-Plasma-M-RT-25 for 25 extractions	
DNA binding buffer	Transparent colorless liquid
Lysis buffer	Transparent colorless liquid
Magnetic Beads, MB	Brown suspension
Wash buffer No. 1	Transparent colorless liquid
Wash buffer No. 2	Transparent colorless liquid

Eluent	Transparent colorless liquid
1.1.2 DNA-Plasma-M-RT-50 for 50 extractions	
DNA binding buffer	Transparent colorless liquid
Lysis buffer	Transparent colorless liquid
Magnetic Beads, MB	Brown suspension
Wash buffer No. 1	Transparent colorless liquid
Wash buffer No. 2	Transparent colorless liquid
Eluent	Transparent colorless liquid
1.1.3 DNA-Plasma-M-RT-50 for 50 extractions with Magnetic separation rack	
DNA binding buffer	Transparent colorless liquid
Lysis buffer	Transparent colorless liquid
Magnetic Beads, MB	Brown suspension
Wash buffer No. 1	Transparent colorless liquid
Wash buffer No. 2	Transparent colorless liquid
Eluent	Transparent colorless liquid
Magnetic separation rack	Holder for 1.5 ml and 15 ml tubes with 100*15*15 mm magnet
1.2 Physical and chemical parameters	
Hydrogen ion concentration, pH	
DNA binding buffer	min 6,0 pH, max 8,0 pH
Wash buffer No. 1	min 6,0 pH, max 8,0 pH
Wash buffer No. 2	min 6,0 pH, max 8,0 pH
1.3. Completeness	In accordance with p. 1.4 TS 21.20.23-010-97638376-2017
1.4. Labeling	In accordance with p. 1.5 TS 21.20.23-010-97638376-2017

1.5. Packaging	In accordance with p. 1.6 TS 21.20.23-010-97638376-2017
2. Performance Characteristics	
2.1. DNA extraction efficiency, %, not less	20
2.2. Purity of DNA isolation, A260/280, not less	1,6

4.3 Clinical Efficiency Characteristics:

Diagnostic sensitivity of the medical device under trial was calculated based on the results of clinical trials by confirming functional property values (DNA extraction efficiency – at least 20%, purity of DNA isolation, A260 / 280 – not less than 1.6) and its efficiency when used in a series of 150 experiments with extracted circulating free DNA samples. The medical device achieved 98.1 % of diagnostic sensitivity and 90% of confidence probability (DNA extracted from blood plasma samples is suitable for subsequent analysis in clinical laboratory diagnostics by allele-specific real time PCR).

5. Risks Associated With the Use of DNA-Plasma-M-RT reagent kit

The border risk zone includes the following:

- loss of functional properties of reagents included in the kit due to transportation, storage or operation under inappropriate conditions;
- contaminants in DNA;
- DNA extraction from insufficient amount of plasma;
- failure to meet requirements for sample preparation, testing and disposal due to the fact that unqualified personnel work with the kit;
- use of an unsuitable kit (use after the expiration date or if the packaging is broken).

In the area of the unacceptable zone, no risks were identified.

Total residual risk of using a medical device “Reagent Kit for Free-Circulating DNA Extraction from Blood Plasma (DNA-Plasma-M-RT)”, produced by TestGene, LLC is acceptable; the benefit of its use exceeds the risk.

6. Precautions When Working With the Kit

Potential risk Class – 2a in accordance with Nomenclature Classification of Medical Devices approved by the Order of the Ministry of Health of the Russian Federation dated June 6, 2012 No.4n.

The work should be carried out in a laboratory that performs molecular biological (PCR) studies of clinical material in compliance with Health Regulations 1.3.2322-08 "Safety of work with microorganisms of pathogenicity group III–IV (hazard) and parasites", SanPiN 2.1.7.2790-10 "Sanitary and epidemiological requirements for medical waste handling" and Methodology Guidelines 1.3.2569-09 "Work of laboratories that use methods of nucleic acid amplification when working with material containing microorganisms of pathogenicity group I-IV".

When working it is required:

▪ to handle test samples as infectious and dangerous, organize work and storage in accordance with Health Regulations 1.3.2322-08 "Safety of work with microorganisms of pathogenicity group III–IV (hazard) and parasites";

▪ to remove and disinfect spilled samples or reagents, using disinfectants in accordance with Health Regulations 1.3.2322-08 "Safety of work with microorganisms of pathogenicity group III–IV (hazard) and parasites";

▪ testing process in a laboratory should be unidirectional. The analysis is performed in separate rooms (zones). Work should start in the Extraction Zone and continue in the Amplification and Detection Zone. Do not return samples, equipment, or reagents to the area where the previous stage of the process is performed;

▪ unused reagents, expired reagents, and used reagents should be disposed in accordance with the requirements of SanPiN 2.1.7.2790-10 "Sanitary and Epidemiological Requirements for Medical Waste Handling";

▪ use and change disposable tips for automatic dispensers with a filter after each operation. Disposable plastic dishes must be removed into a special container containing a disinfectant that can be used for decontamination of medical waste;

▪ table surfaces, as well as the rooms where PCR is performed, must be exposed to UV-radiation for 30 minutes before and after the work is completed;

▪ use the kit strictly for its intended purpose, according to this instruction;

▪ only specially trained personnel is allowed to work with the kit;

▪ not to use the kit after the expiration date;

▪ not to use the reagent kit if the external packaging is broken or the appearance of the reagent does not match the description;

▪ use disposable gloves, lab coats, and eye protection when handling samples and reagents. Wash hands thoroughly after work;

▪ all kit components are non-toxic to humans in the concentrations used. In case of contact with the skin or mucous membranes, the contact area must be washed with plenty of water.

Necessary precautions against influence of magnetic fields, external electrical influence, electrostatic discharges, pressure or pressure drops, overload, or sources of thermal ignition are not recommended.

The kit contains no substances of human or animal origin that have a potential infectious nature, so precautions against any special, unusual risks when using or selling the product are not provided.

7. Required Equipment and Materials

Equipment:

1. Sterile laminar box (e.g., BAVp-01-Laminar-S-1,2, Laminar Systems, Russia),
2. Thermostat for Eppendorf type test tubes from 25°C to 100 °C (e.g., “Thermo 24-15”, Biokom”, Russia),
3. Medical vacuum aspirator with trap flask (e.g., “OM-1”, Ulyanovsk, Russia),
4. Vortex (for example, “TETA-2”, “Biokom”, Russia),
5. Separate set of automatic variable volume dispensers (e.g., “Eppendorf”, Germany),
6. Refrigerator for +2°C to +8 °C with a freezer no higher than minus 16 °C.

Materials and reagents not included in the kit:

1. Ethyl alcohol (95%),

2. Disposable polypropylene screw-cap or leak-tight when closed micro-tubes, 1.5 mL, DNA and DNase free (e.g., Axygen, USA),
3. Disposable polypropylene screw-cap or leak-tight when closed micro-tubes, 15 mL, DNA and DNase free (e.g., Axygen, USA),
4. 15 mL and 1.5 mL test tube racks (e.g., InterLabService, Russia) and pipette tips (e.g., Axygen, USA),
5. Magnetic separation rack for 1.5 mL and 15 mL test tubes (e.g., InterLabService, Russia),
6. Disposable pipette tips for variable 100 µl, 1,000 µl, and 5 µl volume dispensers with an aerosol barrier, DNA and DNase free (e.g., Axygen, USA),
7. Disposable pipette tips for variable 100 µl, 1,000 µl volume dispensers, DNA and DNase free (e.g., Axygen, USA),
8. Isolation or disposable gown coat and disposable gloves,
9. Container with disinfectant.

Measuring equipment is not required when using the kit.

8. Test samples

Before the work, it is required to study Guidelines "Sampling, Transportation and Storage of Clinical Material for PCR-Diagnostics", developed by the Federal State Budgetary Institution of Science Central Research Institute of Epidemiology of Federal service for surveillance on consumers' rights protection and human well-being (Rospotrebnadzor), Moscow, 2012.

Circulating free DNA is extracted from human peripheral venous blood plasma.

8.1 Biological Sample Collection.

Sample Collection.

To obtain plasma, the peripheral venous blood is collected into a test tube with CPDA or EDTA-K2 as an anticoagulant. For mixing blood with an anticoagulant, the test tube should be turned upside down 2-3 times.

Conditions for transportation and storage of source clinical material:

When using tubes with CPDA as an anticoagulant, it is allowed to transport the whole blood to the laboratory for 2 days at a temperature of

4-8 °C. Plasma should be obtained from blood within 48 hours after blood collection.

When using tubes with EDTA-K2 as an anticoagulant, the blood should be delivered to the lab within one hour. Plasma should be obtained from blood within 2-3 hours after blood collection.

ATTENTION! Do not freeze or heat the test tube with blood over 25 °C.

Sample preparation.

Centrifuge the test tube with blood for 10-15 min at 2000-3000g, then carefully collect the top layer of plasma and transfer it to a separate disposable tube, avoiding getting white blood cell clots and layers with red blood cells into collected plasma. Centrifuge the plasma for 15 minutes at 13000g or 10 minutes at 16000g, then collect the top layer in a separate tube without collecting the sediment at the bottom of the tube. The obtained plasma can be used for extraction.

8.2 Interfering substances and limits of use of analytes

The effect of potentially interfering substances on the function of DNA-Plasma-M-RT reagent kit was tested for potentially interfering substances that may occur during human peripheral whole blood collection and during normal use of DNA-Plasma-M-RT reagent kit, and presumably affect the result of extraction of freely circulating DNA from human blood plasma of appropriate quality and amount required for clinical laboratory diagnostics by allele-specific real-time PCR.

Potentially interfering substances that may occur during collection of human peripheral whole blood, get into the analyzed DNA samples and affect the ability of DNA-Plasma-M-RT reagent kit to isolate freely circulating DNA from human blood plasma, and the range of concentrations studied are shown in Table 4.

Table 4 – Potentially interfering substances that may occur during collection of human peripheral whole blood, and their concentrations that inhibit PCR.

Inhibiting substances	Maximum concentration	Minimum concentration
Heparin (anticoagulant)	0,15 U/ml	0,075 U/ml

Sodium citrate (anticoagulant)	1 mmol/l	0,5 mmol/l
Hemoglobin, a high-molecular fraction of protein (hemolysis)	1 мг/мл	0,5 мг/мл
Triglycerides (chylous)	0,5 ммоль/л	0,25 ммоль/л

To study potentially interfering substances in the normal use of DNA-Plasma-M-RT reagent kit, ethyl alcohol (95%) is selected as an interfering substance (due to its potential inhibitory effect on PCR), which is added to Washing Buffer No. 1 and Wash Buffer No. 2 at the stage of preparing the components for the study. Concentrations of potentially interfering substance are shown in Table 5.

Table 5 – Concentration range of interfering substances studied during the trials

Inhibiting substance	Maximum concentration (mcl/ 200 mcl of DNA buffer)	Minimum concentration (mcl/ 200 mcl of DNA buffer)
Ethyl alcohol (95%)	$1,35*10^{-3}$	$3,38*10^{-4}$

To assess the effect of potentially inhibiting substances, a study was performed by analyzing their effects in two concentrations (maximum and minimum), the range of which is expected to occur during the procedure of collecting human peripheral whole blood and during normal use of DNA-Plasma-M-RT reagent kit, on the values of purity of DNA extraction (expressed in relation to optical densities of extracted DNA buffer, A260/280) and DNA extraction efficiency (in %), followed by allele-specific real time PCR analysis.

Based on the obtained results, substances present in DNA samples can have an interfering effect at concentrations exceeding the permissible ones:

Inhibiting substances	**Concentrations inhibiting PCR**
Heparin (anticoagulant)	$\geq$ 0,15 U/ml
Sodium citrate (anticoagulant)	>1 mmol/L

Hemoglobin, a high-molecular fraction of protein (hemolysis)	≥ 1 mg/ml
Triglycerides (chylous))	>0,5 mmol/L

According to the results, ethyl alcohol, studied in concentrations expected to occur with normal use of the reagent kit does not affect the ability of DNA-Plasma-M-RT reagent kit to extract freely circulating DNA (cfDNA) from blood plasma in quality and amount appropriate for testing in clinical laboratory diagnostics by real-time allele-specific PCR.

8.3 Restrictions on the use of the tested material:

To obtain plasma, the blood is collected into a test tube with CPDA or EDTA-K2 as an anticoagulant. When using tubes with CPDA as an anticoagulant, it is allowed to transport whole blood to the laboratory for 2 days at a temperature of 4-8 °C. When using tubes with EDTA-K2 as an anticoagulant, plasma should be obtained from blood within 2-3 hours after blood collection.

- Blood freezing is not allowed before plasma preparation.
- It is not allowed to work with hemolysed and chylous blood, when testing such samples, false results may be obtained.
- When obtaining plasma, it is not allowed to contaminate it with blood cells.
- Only a single freezing thawing of the resulting plasma is allowed.
- After completion of DNA extraction the PCR analysis should be immediately started.
- Personnel errors when collecting blood, obtaining plasma and during DNA extraction, violation of the recommended instructions may lead to false results.

8.4 Possible conditions for test samples storage

Conditions for transportation and storage of source clinical material:

When using tubes with CPDA as an anticoagulant, it is allowed to transport the whole blood to the laboratory for 2 days at a temperature of 4-8 °C. Plasma should be obtained from blood within 48 hours after blood collection.

When using tubes with EDTA-K2 as an anticoagulant, the blood should be delivered to the lab within one hour. Plasma should be obtained from blood within 2-3 hours after blood collection.

ATTENTION! Do not freeze or heat the test tube with blood over 25 °C.

Plasma storage conditions**:**

- at temperatures ≤ 4−8 °C — up to 5 days;
- at - 20°C — during one month;
- at - 70 °C — for a long time.

ATTENTION! Plasma can be frozen-thawed only once.

ATTENTION! It is not allowed to work with hemolysed and chylous blood. When testing such samples, it is possible to get false results.

Storage conditions for a circulating free DNA (cfDNA) sample extracted from blood plasma:

Obtained DNA should be stored at temperatures from +2 to +8ºC within 12 hours before testing, at - 20°C – max 3 months or at -70 °C – for one year.

9. Preparation of the components for testing

It is not required to install, assemble, adjust, or calibrate a medical device before operation.

If the plasma sample was stored in the refrigerator, warm up the plasma to room temperature before DNA extraction.

!!! The suspension of magnetic beads is two-phase, easily and quickly forms clearly separated two phases. Before the work starts and before each operation with magnetic bead buffer, completely resuspend the magnetic bead buffer on vortex or by pipetting.

Layering or precipitation of crystalline sediment does not affect the quality of buffers. In case of crystalline precipitate or layering of components it is necessary to warm the bottles at 50 °C and mix thoroughly until the precipitate is completely dissolved and the buffers are homogenized.

All components of the kit must be thoroughly mixed before starting work.

Before operating, prepare **“Wash buffer No.1” and “Wash buffer No.2”**.

When using **DNA-Plasma-M-RT-25**:

1) Add 8 ml of ethyl alcohol (95%) to Wash buffer No.1.

2) Add 32 ml of ethyl alcohol (95%) to Wash buffer No.2.

Mark the labels of the bottles on completion of operation.

For **DNA-Plasma-M-RT-50 and DNA-Plasma-M-RT-50 with Magnetic separation rack:**

1) Add 15 ml of ethyl alcohol (95%) to Wash buffer No.1.

2) Add 60 ml of ethyl alcohol (95%) to each bottle with Wash buffer No.2.

Mark the labels of the bottles on completion of operation.

10. Testing procedure

Only specially trained personnel with PCR analysis skills are allowed to work with the kit.

Extraction protocol can be modified to scale up if it is necessary to get more of the final material:

Plasma amount	Lysis buffer amount, µl	DNA biding buffer amount, µl	Magnetic beads amount, µ	Wash buffer No.1 amount, µl	Wash buffer No.2 amount, µl
2 ml	600	3 000			
3 ml	900	4 500			
4 ml	1 200	6 000			
5 ml	1 500	7 500	20	700	700

DNA is extracted from 2 ml of plasma sample.

For each sample, prepare two 15 ml test tubes and two 2 or 1.5 ml test tubes.

Mark test tubes corresponding to samples.

1. Add 2 ml plasma and 600 µl of lysis to 15 ml test tube, vortex.
2. Incubate at room temperature for 5 min, mixing repeatedly.
3. Mix 3 ml of binding buffer and 20 µl of magnetic beads in a separate 15 ml test tube, vortex.
4. Add magnetic bead suspension to the test tube with plasma (step1), vortex.

5. Incubate at room temperature for 15 minutes, vortex.

6. Move the test tube into Magnetic separation rack, wait while beads are captured on the test tube walls (usually for 5 – 10 minutes), discard supernatant.

7. Add 700 µl of Wash buffer No.1 to the test tube. Thoroughly resuspend magnetic beads in Wash buffer No.1 by pipetting and move the obtained suspension of magnetic beads to 1.5 ml test tube.

8. Move the test tube to the Magnetic separation rack, leave until beads are captured on the walls of the test tube, discard supernatant (usually 1 minute is required).

9. Add 700 µl of Wash buffer No.2 to the test tube, thoroughly vortex. Remove drops by short time centrifuging.

10. Place the test tube into a Magnetic separation rack, leave until beads are captured on the walls of the test tube, and completely discard supernatant (usually 1 minute is required).

11. Repeat steps 9 and 10.

12. Place the test tube with slightly-opened cap into a thermostat and incubate at 60 °C for10 minutes to dry and remove residual ethanol.

Make sure that the residual ethanol is completely removed!

13. Add 60 ml of eluent to the test tube. Carefully resuspend the beads by pipetting.
14. Incubate in a thermostat at 60 °C for 10 minutes.
15. Place the test tube into a Magnetic separation rack, wait until the particles are completely captured on the test tube wall, and transfer the supernatant containing the extracted DNA to a new test tube.

When carrying out PCR it is recommended to keep the test tube with extracted DNA in a Magnetic separation rack.

If necessary, the volume of the eluent can be increased, but the concentration of DNA will decrease. The concentration of DNA can be increased by reducing the volume of eluent.

ATTENTION! If the purpose of DNA extraction is to analyze fetal DNA present in the maternity blood, the PCR reaction should begin immediately after the end of extraction. Fetal DNA is present in the mother's blood in very low concentrations and in a degraded state. During storage, fetal DNA can be destroyed, which can lead to false negative results.

11. Possible problems and their buffers

1. <u>Low yield of DNA, cause and possible buffer</u>:

▪ state of the sample (the sample contains an insufficient quantity of DNA; the sample was long stored, or improperly stored, or repeatedly subjected to the procedure of freezing and thawing) – possible buffers: take more of the source material or perform elution in a smaller amount of the buffer; repeat collection of the material;

▪ magnetic beads are poorly captured on the Magnetic separation rack, it makes impossible to discard supernatant after incubation (step 6), supernatant is cloudy – warm the test tube at 60^0C until the buffer is clear. Warm up the plasma stored in the refrigerator to room temperature before DNA extraction.

▪ incomplete drying of beads before adding eluent – remove Wash buffer No.2, increase drying time after removing Wash buffer No.2;

▪ overdrying of magnetic beads – dry magnetic beads for 10 minutes;

▪ incomplete lysis - after adding lysing buffer, suspend the sample as thoroughly as possible;

▪ large amount of eluent - select the optimal eluent size to obtain the desired DNA concentration.

2. <u>Protein contamination</u> – it is necessary to achieve the most thorough suspension of magnetic beads.

3. <u>Possible degradation of DNA</u>, cause and possible buffer: an old sample, or the sample was subjected to freezing-thawing – it is necessary to collect the material again. Avoid freezing the sample during transport and storage.

If you have any questions or need advice, please contact TestGene support service - see section 14.

12. Storage, transportation and usage conditions

Storage.

DNA-Plasma-M-RT reagent kit in the manufacturer's package should be stored at a temperature not higher than +30 °C and relative humidity up to 90%. Atmospheric pressure is not controlled, because it does not affect the quality of the product.

A reagent kit stored in violation of storage conditions cannot be used.

Transporting.

DNA-Plasma-M-RT reagent kits can be transported by all types of covered vehicles in accordance with the transport rules applicable to this type of transport. It is allowed to transport the kit at a temperature of max +30°C, and relative humidity up to 90%. Atmospheric pressure is not controlled, because it does not affect the quality of the product.

A reagent kit transported in violation of temperature conditions cannot be used.

Shelf Life. Shelf life for DNA-Plasma-M-RT reagent kit is 12 months from the date of acceptance by the manufacturer's Quality Control Department, provided that all conditions of transportation, storage and operation are observed. A reagent kit with expired shelf life cannot be used.

Expiration Date of Opened Kit Components – is 12 months from the date of acceptance by the manufacturer's Quality Control Department, provided that the kit is stored at temperature max +30°C. After opening the bottles and adding ethyl alcohol (95%) to Wash buffer No. 1 and Wash buffer No.2 the shelf life of the kit is 6 months.

A reagent kit stored in violation of storage conditions cannot be used.

13. Disposal

Reagent kits that have become unusable, including shelf life expiration, are subject to disposal in accordance with SanPiN 2.1.7.2790-

10 requirements “Sanitary and Epidemiological Requirements for Medical Waste Handling”.

According to medical waste classification, the kits belong to Class A (epidemiologically safe waste close in composition to solid household waste). Unused reagents in accordance with paragraph 4.28 of SanPiN 2.1.7.2790-10 “Sanitary and Epidemiological Requirements for Medical Waste Handling” are collected in a single-use labeled packaging of any color (except yellow and red).

Test tubes and materials after the use are disposed in accordance with Methodology Guidelines 287-113 (Methodology Guidelines for Disinfection, Pre-Sterilization Cleaning and Sterilization of Medical Devices).

Liquid components (reagents) are disposed by draining into a sewer with preliminary dilution of a reagent with tap water 1: 100 and removing the remains of packages as industrial or household garbage.

Consumer packaging of DNA-Plasma-M-RT reagent kit is subject to mechanical destruction with the removal of residues as industrial or household garbage.

Personnel disposing reagent kits must comply with the safety rules for conducting a particular method of disposal.

14. Warranty Obligations, Contacts

The manufacturer guarantees that DNA-Plasma-M-RT reagent kit meets technical specification requirements in compliance with established requirements for transportation, storage and operation.

In case of complaints about the reagent kit quality, undesirable events or incidents, submit information to:

Limited Liability Company “TestGene”
(TestGene, LLC),
9 44th Inzhenerny Proezd, office 13, Ulyanovsk 432072
Tel.: +7 (499) 705-03-75
www.testgen.ru

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

European Authorized Representative:
CMC MEDICAL DEVICES & DRUGS S.L.
C/ Horaclo Lengo No. 18, CP 29006
Malaga, Spain
Phone: +34 951 214 054
Fax: +34 952 330 100
E-mail: info@cmcmedicaldevices.com