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INSTRUCTION

Kit for DNA isolation from blood plasma (DNA-Plasma-M)
by TS 9398-002-97638376-2015 in the versions:
1) "DNA-Plasma-M-50" for 50 preparations,
2) "DNA-Plasma-M-100" for 100 preparations

Version 2 – revised on 29/12/2016

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1. INTENDED USE

Kit for DNA isolation from blood plasma (DNA-Plasma-M) by TS 9398-002-97638376-2015 in the versions: 1) "DNA-Plasma-M-50" for 50 preparations, 2) "DNA-Plasma-M-100" for 100 preparations is intended for isolation of free-circulating DNA from blood plasma.

DNA isolation is based on reversible binding of nucleic acids on magnetic microbeads surface.

Clinical relevance is ensuring of pre-analytical testing phase of free-circulating DNA. Purified DNA well-suitable for noninvasive testing methods in clinical pathology.

Application area – oncology, prenatal testing.

Material – whole blood plasma (fresh or frozen).

Description.

The most volume of DNA and RNA in the organism is located inside cells. Small amount of nucleic acids can be found circulating in blood. These DNA and RNA molecules originate from dead cells that released its contents to bloodstream. Presence of free circulating DNA and RNA allows using noninvasive testing methods for a wide range of clinical conditions and diseases. Detection of specific DNA and/or RNA provides a unique opportunity for early diagnostics of different pathologies. It includes early stages of cancer, many viral infections, noninvasive prenatal diagnostics during pregnancy period.

Indication. Kit «DNA-Plasma-M» is recommended to use for testing in clinical pathology: sex determination, detection of gene carrier with congenital defect, chromosome pathologies during DNA testing; for testing of mutations in circulating DNA and detection of viral nucleic acids in human blood plasma.

The kit is intended for professional use only in medical centers and laboratories.

Total time of DNA isolation procedure from 1 sample is 90 minutes.

It is possible to perform DNA isolation from more than 1 ml of plasma to get more amount of DNA finally. In this case it is necessary to proportionally change volumes of reagents.

2. KIT CHARACTERISTICS

Kit «DNA-Plasma-M» in the versions:

1) «DNA-Plasma-M-50» for 50 preparations;
2) «DNA-Plasma-M-100» for 100 preparations.

Kits for 50 and 100 preparations include:

Reagent	Description	Quantity	
		«DNA-Plasma-M-50»	«DNA-Plasma-M-100»
DNA Binding buffer	Transparent colorless liquid	1 bottle 90 ml	2 bottles 90 ml each
Proteinase K	White powder	1 bottle 50 mg	1 bottle 100 mg
Proteinase K Diluent	Transparent colorless liquid	1 bottle 2,5 ml	1 bottle 5 ml

Magnetic microbeads	Brown liquid	1 tube 1,2 ml	2 tubes 1,2 ml each
Wash buffer №1	Transparent colorless liquid	1 bottle 30 ml	1 bottle 60 ml
Wash buffer №2	Transparent colorless liquid	1 bottle 15 ml	2 bottles 15 ml each
Eluent	Transparent colorless liquid	1 bottle 5 ml	1 bottle 10 ml

Calibrators and control materials are not used in the kit.
Note: sample does not contain any other ingredients that may influence the procedure.

OPERATING PRINCIPLE

The principle of the method is based on DNA reversible binding on the magnetic particles coated with a special surface. After the sample is lysed, nucleic acids contained in it are bound to the magnetic microbeads, they must be washed with buffers from the kit. After the wash cycles are finished, the pellet of magnetic microbeads must be dried and DNA can be eluted.

Method limitations:

If fetal DNA testing in mother's blood is the purpose of isolation, there are specified raise requirements to blood drawing, storage, plasma obtaining and storage. Inaccurate results may be obtained because of disruption of blood cells and intracellular DNA release.

- For plasma obtaining blood must be taken into tube with EDTA or CPDA. If tube with CPDA is used as anticoagulant, it is permissible to transport whole blood during 2 days without freezing. If EDTA is used, it is necessary to separate plasma during 3-4 hours since blood drawing.
- It is not permitted to freeze blood before plasma isolating.
- It is not permitted to use laky and chylous blood. Using such samples may cause inaccurate results while testing.
- It is not permitted to contaminate plasma with blood cells.
- Isolated plasma may be frozen and thawed only once.
- After DNA isolation you should start PCR. Fetal DNA is in mother's blood in low concentration and fragmented.
- Operator mistakes during blood drawing, plasma obtaining and DNA isolation, violation of recommended instruction may lead to getting of inaccurate results.

3. Analytical characteristics

DNA Extraction Efficiency, %, not less than	20
Purity of extracted DNA, A260/280, not less than	1,6

4. Safety precautions

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:

- 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».
- 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».
- 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.
- 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».
- 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.
- Uviolize table surfaces, rooms for PCR during 30 minutes before and after work completion.
- Use kit by designation according to this instruction.
- Admit only specially trained staff to the work with the kit.
- Do not use kit after the expiry date.
- Use disposal gloves, lab coats, protect your eyes while working with the samples and reagents. Wash hands thoroughly at the end of the work.
- Avoid contact with skin, eyes and mucous membranes. In case of contact, wash immediately the affected area with water and seek medical attention.
- 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. While working with biological material samples it is necessary to conform with 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».

5. Equipment and materials

Equipment:

- Sterile laminar box (e.g., BAVp-01-Laminar-S-1,2, Laminar Systems, Russia)
- Thermostat for Eppendorf tubes from 25 to 100 °C (e.g., TERMO 24-15, Biokom, Russia)
- Vacuum ejector with trapping flask (e.g., OM-1, Russia)
- Magnetic rack for Eppendorf tubes for 1,5-2 ml
- Vortex (e.g., TETA-2, Biocom, Russia)
- Certain kit of variable volume pipettes
- Refrigerator from 2 °C to 8 °C
- Freezer from – 2 °C to – 40 °C.

Materials and reagents not included in the kit:

- Absolute ethanol (95%)
- Disposal polypropylene microtubes for 1,5 ml, screw-capped or closed tightly, DNA and DNAse-free (e.g., Axygen, USA)
- Racks for microtubes for 1,5 ml (e.g., InterLabService, Russia) and tips (e.g., Axygen, USA)
- 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)
- Disposal tips for variable volume pipettes for up to 100 µl and 1000 µl, DNA-free and DNAse-free (e.g., Axygen, USA)
- Disposal or individual coats and disposal gloves
- Containers with disinfection solution.

6. Test Samples

Material for testing is whole blood plasma (fresh or frozen).

Obtaining of biological material.

Place the blood sample into a tube with EDTA or CPDA.

Mix tube content by turning around.

If CPDA is used as anticoagulant, the samples of whole blood can be transported without freezing for 2 days. If EDTA is used, plasma should be separated during 3-4 hours after blood collection.

Sample preparation.

Centrifuge tube with blood 10-15 min at 2000-3000g. Collect plasma layer and transfer it into new disposal tube carefully. Avoid taking buffy coats and erythrocytes layers. Centrifuge plasma 15 min at 13000g or 10 min at 16000g. Collect plasma layer and transfer it to new disposal tube. Do not disturb the precipitate at the bottom of tube. Obtained plasma may be used for extraction.

It is recommended to take not less than 1 ml of plasma and to dilute in 50-80 µL (final volume should be the minimal required for one testing – this guarantee the highest DNA yield).

If EDTA is used the plasma sample should be delivered to the laboratory within 16-24 hours after obtaining (it is recommended to force delivery and use cold box at -20°C).

Plasma samples should be stored:

- at temperature not above 2−8 °C – for 5 days;
- at temperature not above -20°C – for 1 month;
- at temperature -70 °C – for long storage.

ATTENTION! Only one freeze-thaw cycle is allowed.

ATTENTION! Do not work with laky and chylous blood. It may cause wrong results of testing.

Safety precautions during working with testing material – see Section 4.

7. PREPARATION OF THE COMPONENTS FOR TESTING

The suspension with magnetic microbeads forms two separate phases. Resuspend the solution with magnetic microbeads thoroughly by pipetting up and down or by Vortexing before starting each manipulation with the magnetic microbeads.

Layering and precipitation have no impact on quality of the solutions. In case of layering or precipitation, it is necessary to heat components at 50 °C and mix thoroughly to achieve complete resolution and homogenization of the solutions.

It is necessary to mix all components thoroughly before starting work.

Before starting work, prepare the following:

•**Proteinase K.**

Add the whole volume of Proteinase K Diluent to lyophilized Proteinase K and dissolve Proteinase K.

• **Wash buffer №1.**

Add **15 ml of ethanol (95%) to Wash Buffer №1** for kit «DNA-Plasma-M-50» or **30 ml of ethanol (95%)** for kit «DNA-Plasma-M-100».

•**Wash buffer №2.**

Add **60 ml of ethanol (95%) to each bottle of Wash Buffer №2.**

8. Testing procedure

Admit only specially trained staff to the work.

Isolation procedure is sketched on Figure 1.

DNA isolation from **1000 µl of plasma sample**.

Prepare **4 tubes for 1.5 or 2 ml** for each sample.

Mark the tubes according to the samples.

(Mix the reagents thoroughly before each adding).

1. Transfer **25 µl of proteinase K,** then **500 µl of sample** and **500 µl of DNA Binding buffer** into each of two tubes (1.5 or 2 ml).

2. Vortex the mixtures thoroughly (mix 5 times for 5-10 seconds each tube and precipitate drops from caps by centrifugation)

3. Incubate the tubes **at 60°C for 15 min**. During incubation mix each tube at vortex every 5 minutes for 5 seconds.

4. After incubation place **500 µl** of well-stirred **DNA Binding buffer** and **20 µl** of well-stirred **buffer with magnetic microbeads** into separate tube. Mix 20-30 times the buffer with magnetic microbeads by pipetting up and down.

5. For each sample tube take **260 µl** of the **suspension of magnetic microbeads in DNA binding buffer**. Mix it 30-40 times by pipetting up and down.

6. Incubate the samples **at room temperature for 5 min**. After incubation vortex for 3-5 seconds each tube, precipitate drops from caps by centrifugation (Centrifuge not longer than 1 second. Tubes must be placed so that cap loop is outward).

7. Place tubes in a magnetic rack, and wait until the microbeads gather on the tube walls (usually it takes 10 min) and **discard the supernatant** not taking out from rack by pipette or using suction unit.

8. Add to one of two tubes referring to the same sample **700 µl** of well-stirred **wash buffer №1**, completely resuspend magnetic microbeads in the buffer 15-20 times by pipetting up and down. **Transfer the resulting suspension of magnetic microbeads and wash buffer №1 into the second tube** with sediment of magnetic microbeads referring to the same sample. Thoroughly resuspend magnetic microbeads 20-30 times by pipetting up and down.

9. Place tube in a magnetic rack, wait (1-2 min) and discard the supernatant not taking out from rack by pipette or using suction unit.

10. Add to the tube **700 µl** of well-stirred **wash buffer №2** and resuspend thoroughly magnetic microbeads 20-30 times by pipetting up and down.

11. Place tube in the magnetic rack, wait until the microbeads completely gather on the tube wall (1-2 min) and discard the supernatant not taking out from rack by pipette or using suction unit.

12. Repeat the 10&11 steps.

13. Place the tubes with opened caps into the thermostat and incubate **at 60 °C for 10 min** to dry and to discard residual ethanol (95%).

14. Add to the tube **60 µl of Eluent**. Thoroughly resuspend the microbeads 20-30 times by pipetting up and down.

15. Incubate **at 60°C for 10 min**. Shake 2-3 times during incubation.

16. Place the tube into the magnetic rack, wait until the microbeads completely gather on the tube wall.

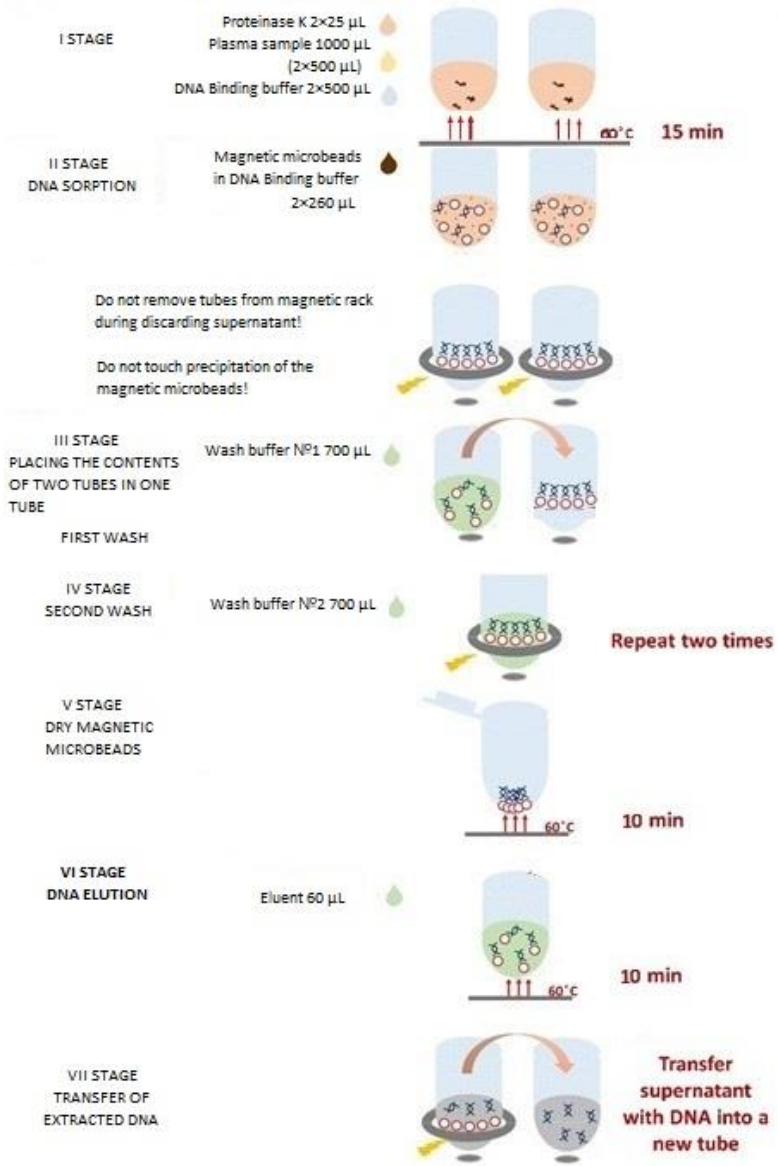
17. **Carefully transfer supernatant with DNA into a new tube without touching the magnetic microbeads by pipette tips.**

Note! The transferring of the extracted DNA is performed without removing the tubes from the magnetic rack. During pipetting of extracted DNA don't remove tube from magnetic rack.

ATTENTION! If fetal DNA testing in mother's blood is the purpose of isolation, it is necessary to start PCR-reaction after finish of isolation. Fetal DNA is in mother's blood in low concentration and fragmented. Fetal DNA can be destroyed during storage that leads to false negative results.

If necessary, obtained DNA must be stored:

- at +4 °C – for 1 day,
- at minus 20 – minus 40°C – not more than month,
- at minus 86 °C – for long storage.


I STAGE
Proteinase K 2×25 µL
Plasma sample 1000 µL
(2×500 µL)
DNA Binding buffer 2×500 µL
60°C
15 min
II STAGE
DNA SORPTION
Magnetic microbeads
in DNA Binding buffer
2×260 µL
Do not remove tubes from magnetic rack during discarding supernatant!
Do not touch precipitation of the magnetic microbeads!
III STAGE
PLACING THE CONTENTS OF TWO TUBES IN ONE TUBE
FIRST WASH
Wash buffer №1 700 µL
IV STAGE
SECOND WASH
Wash buffer №2 700 µL
Repeat two times
V STAGE
DRY MAGNETIC MICROBEADS
60°C
10 min
VI STAGE
DNA ELUTION
Eluent 60 µL
60°C
10 min
VII STAGE
TRANSFER OF EXTRACTED DNA
Transfer supernatant with DNA into a new tube

9. POSSIBLE PROBLEMS AND ITS SUGGESTIONS

1. **Low DNA yield**, cause and possible suggestion:
   - State of sample (low concentration of DNA in the samples, the samples were long or improperly stored or repeatedly freezed and thawed) – suggestion: take more starting material or use fewer amount of the eluent, resampling of the clinical material;
   - Incomplete drying of the microbeads before adding the eluent – remove the wash solution №2 fully; after incubation check the magnetic microbeads the presence of ethanol residue. Light brown color of magnetic microbeads indicates that ethanol is fully evaporated **(It is important that microbeads are completely dried).**
   - Incomplete lysis – suggestion: the sample has to be suspended thoroughly after addition of DNA binding buffer;
   - A large volume of elution buffer – suggestion: find the best volume of eluent to obtain the desired DNA concentration.
2. **Protein contamination** - make sure the magnetic microbeads are thoroughly suspended at all steps.
3. **DNA degradation** – cause and possible suggestion – old sample or sample were subjected to freezing and thawing – it is necessary to collect material again. Avoid sample freezing during transportation and storage.

If you have any questions, please contact support service of TestGene – see Section 12.

10. STORAGE, TRANSPORTATION AND USAGE CONDITIONS

Storage. Store kit at temperature not above than +30 °C and relative humidity up to 90%. Atmosphere pressure is not controlled because it does not influence the sample quality.

Store Proteinase K at temperature minus 20 °C.

Shelf life of opened components – 12 months providing store at temperature not above than +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 with violations are not to be used.

Transportation. Transport the kit at temperature not above +30 ºC and relative humidity up to 90%. Atmosphere pressure is not controlled because it does not influence the sample quality.

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

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

11. UTILIZATION

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».

It is permitted to use sample components (with correct operational properties) included in the unusable kit within the expiry date.

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-Plasma-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.

12. WARRANTY OBLIGATIONS, CONTACT INFORMATION

The manufacturer guarantees stable operation of the kit 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

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