902 2025-02-13

For professional use only

PREP-MB-DBS DWP DNA Extraction Kit

INSTRUCTION FOR USE

EC REP

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REF

P-128-N/9EU
P-128-P/9EU
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902.2025.02.13

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1. INTENDED USE

The **PREP-MB-DBS DWP DNA Extraction Kit** is intended for semi-automatic and automatic human genomic DNA extraction from dried blood spots for subsequent polymerase chain reaction (PCR) analysis.

This medical device is an auxiliary agent for *in vitro* diagnosis.

The application of the kit does not depend on population and demographic aspects. There are no contradictions for use of the **PREP-MB-DBS DWP DNA Extraction Kit.**

The **PREP-MB-DBS DWP DNA Extraction Kit** can be used in clinical and diagnostic laboratories of medical institutions and research practice.

Potential users: personnel qualified in molecular diagnostics methods and working in the clinical and diagnostic laboratory.

It is necessary to apply the kit only as directed in this instruction for use.

2. METHOD

The method is based on lysis and release of nucleic acids under the action of a chaotropic agent (guanidine thiocyanate) with subsequent sorption on paramagnetic nanoparticles and clearing from impurities.

3. CONTENT

The **PREP-MB-DBS DWP DNA Extraction Kit** is produced in the following packages: package N and package P (Set No. 1, Set No. 2).

The **PREP-MB-DBS DWP DNA Extraction Kit** content is represented in Tables 1-3.

Table 1. The **PREP-MB-DBS DWP DNA Extraction Kit** content, package N, for P-128-N/9EU

Reagent	Description	Total volume	Amount
Lysis solution	Slightly foamy colorless transparent liquid	28.8 mL	1 vial
Sorbent	Liquid with precipitate forming brown suspension upon shaking	24 mL	1 vial
Wash solution No. 1	Slightly foamy blue transparent liquid	28.8 mL	1 vial
Wash solution No. 2	Colorless transparent liquid	67.2 mL (33.6 mL in each vial)	2 vials
Elution solution	Colorless to pink transparent liquid	6.72 mL	1 vial

Table 3. The **PREP-MB-DBS DWP DNA Extraction Kit** content, package P, Set No. 1, for P-128-P/9EU

Reagent	Description	Total volume	Amount
Lysis solution	Slightly foamy colorless transparent liquid	28.8 mL	1 vial
Sorbent	Liquid with precipitate forming brown suspension on shaking	24 mL (250 µL in each well)	1 96 deep-well plate
Wash solution No. 1	Slightly foamy blue transparent liquid	28.8 mL (300 µL in each well)	1 96 deep-well plate
Wash solution No. 2	Colorless transparent liquid	67.2 mL (700 µL in each well)	1 96 deep-well plate
Elution solution	Colorless or slightly pink transparent liquid	6.72 mL (70 µL in each well)	1 96 deep-well plate
96 deep-well plate for pretreated samples	1 pc		
96 deep-well plate*	1 pc		
96 tip comb	1 pc		
Plate sealing film**	1 pc		
* - to place tips onto magnetic rods ** - to seal the plate with extracted nucleic acids for storage			

Table 4. The **PREP-MB-DBS DWP DNA Extraction Kit** content, package P, Set No. 2, for P-129-P/9EU

Reagent	Description	Total volume	Amount
Lysis solution	Slightly foamy colorless transparent liquid	28.8 mL (300 µL in each well)	1 96 deep-well plate
Sorbent	Liquid with precipitate forming brown suspension on shaking	24 mL (250 µL in each well)	1 96 deep-well plate
Wash solution No. 1	Slightly foamy blue transparent liquid	28.8 mL (300 µL in each well)	1 96 deep-well plate
Wash solution No. 2	Colorless transparent liquid	67.2 mL (700 µL in each well)	1 96 deep-well plate
Elution solution	Colorless or slightly pink transparent liquid	6.72 mL (70 µL in each well)	1 96 deep-well plate
96 deep-well plate*	1 pc		
96 tip comb	1 pc		
Plate sealing film**	1 pc		
* - to place tips onto magnetic rods ** - to seal the plate with extracted nucleic acids for storage			

All components are ready to use and do not require additional preparation for operation.

The kit is intended for single use and is designed for DNA extraction from 96 analyzed samples (including 95 DBS samples and one negative control).

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- Biological safety cabinet class II;
- Refrigerator with freezer;
- High speed centrifuge (RCF(g) at least 150) with bucket rotor and adapter for 96 deep-well plates (except package N REF P-128-N/9EU);
- Single channel pipettes (dispensers covering 20.0-1000 µL volume range);
- 8-channel pipettes (dispensers covering 30.0 - 300 µL volume range);
- RNase and DNase free filtered pipette tips (volume 200 µL, 300 µL, 1000 µL);
- Pipette rack;
- Automatic or hand puncher for punching 3.0-3.2 mm discs from DBS samples;
- System for automatic nucleic acid extraction in 96 Deep-Well Plate (for example, Auto-Pure 96 (Hangzhou Allsheng Instrument Co., LTD, China));
- 96-well deep-well 2.2 mL plate (for placing and pretreatment of DBS discs);
- 96 deep-well 2.2 mL plates (6 pcs) (for package N, REF P-128-N/9EU);
- thermal seal for 96-well microplates (for package N, REF P-128-N/9EU);
- 96 tip comb (for package N, REF P-128-N/9EU);
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

5. TRANSPORT AND STORAGE CONDITIONS

Expiry date – 12 months from the date of production.

The **PREP-MB-DBS DWP DNA Extraction Kit** must be transported in thermoboxes with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C inside the thermoboxes.

The kit must be transported in the upright position in accordance with the handling sign “TOP”.

Kits transported with violation of temperature conditions must not be used.

All components of the **PREP-MB-DBS DWP DNA Extraction Kit** must be stored at temperatures from 2 °C to 25 °C and out of light over the storage period.

Store in the upright position in accordance with the handling sign “TOP”.

When stored in refrigerator (from 2 °C to 8 °C), a minor precipitate is allowed in lysis solution and wash solution No. 1.

Shelf-life of the kit following the first opening of the primary container: the components of the kit must be stored at temperatures from 2 °C to 25 °C and out of light over the storage period.

The kit must be stored in the upright position in accordance with the handling sign “TOP”.

The kit stored under undue regime must not be used.

An expired **PREP-MB-DBS DWP DNA Extraction Kit** must not be used.

We strongly recommend to follow the given instructions in order to obtain accurate and reliable results.

Conformity of the **PREP-MB-DBS DWP DNA Extraction Kit** to the prescribed technical requirements is

subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

6. WARNINGS AND PRECAUTIONS

Only personnel trained in the methods of molecular diagnostics and the rules of work in the clinical and diagnostic laboratory are allowed to work with the kit.

Handle and dispose all biological samples, reagents and materials used to carry out the assay as if they were able to transmit infective agents. The samples must be exclusively employed for certain type of analysis. Samples must be handled under a laminar flow hood. Tubes containing different samples must never be opened at the same time. Pipettes used to handle samples must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. The reagents must be handled under a laminar flow hood. The reagents required for amplification must be prepared in such a way that they can be used in a single session. Pipettes used to handle reagents must be exclusively employed for this specific purpose. The pipettes must be of the positive dispensation type or be used with aerosol filter tips. The tips employed must be sterile, free from the DNases and RNases, free from DNA and RNA. Avoid direct contact with the biological samples reagents and materials used to carry out the assay. Wear powder-free surgical gloves. Wear protective clothing (work clothes and personal protective equipment) working with microorganisms classified as particularly pathogenic. The protective clothing and personal protective equipment must comply with the work to be performed and health and safety requirements. Avoid producing spills or aerosol. Any material being exposed to biological samples must be treated for at least 30 minutes with disinfecting solution or autoclaved for 1 hour at 121 °C before disposal.

Molecular biology procedures, such as nucleic acids extraction, reverse transcription, PCR-amplification and detection require qualified staff to avoid the risk of erroneous results, especially due to the degradation of nucleic acids contained in the samples or sample contamination by amplification products.

All the liquid solutions are designed for single use and can not be used more than once in amplification reactions. Plastic tubes do not contain phthalates. Do not breathe gas/fumes/vapor/spray produced by the components of the kit. Do not eat/drink components of the kit. Avoid contact with eyes. Only use the reagents provided in the kit and those recommended by manufacturer. Do not mix reagents from different batches. Do not use reagents from third party manufacturers' kits. All laboratory equipment, including pipettes, test tube racks, laboratory glassware, lab coats, bouffant caps, etc., as well as reagents should be strictly stationary. It is not allowed to move them from one room to another. Equip separate areas for the extraction/preparation of amplification reactions and for the amplification/detection of amplification products. Never introduce an amplification product in the area designed for extraction/preparation of amplification reactions. Wear lab coats, gloves and tools, which are exclusively employed for the extraction/preparation of the amplification reaction and for the amplification/detection of the amplification products. Never transfer lab coats, gloves and tools from the area designed for amplification/detection of the amplification products to the area designed for extraction/preparation of amplification reactions. Remove waste materials (tubes, tips) only in a special closed container containing a disinfectant solution. Work surfaces, as well as rooms where NA extraction and PCR are performed, must be irradiated with bactericidal irradiators for 30 minutes before and after the work.

Waste materials are disposed of in accordance with local and national standards. All surfaces in the laboratory (work tables, test tube racks, equipment, etc.) must be treated daily with disinfecting solution.

Emergency actions

Eye Contact: If any component of this kit enters the eyes, wash eyes gently under potable running water for 15 minutes or longer, making sure that the eyelids are held open. If pain or irritation occurs, obtain medical attention.

Skin Contact: If any component of this kit contacts the skin and causes discomfort, remove any contaminated clothing. Wash affected area with plenty of soap and water. If pain or irritation occurs, obtain medical attention.

Ingestion: If any component of this kit is ingested, wash mouth out with water. If irritation or discomfort occurs, obtain medical attention.

Do not use the kit:

- When the transportation and storage conditions are breached;
- When the reagents' appearance does not respond to the kit passport;
- When the kit components packaging is breached;
- After the expiry date provided.

Significant health effects are **NOT** anticipated from routine use of this kit when adhering to the instructions listed in the current manual.

7. SAMPLES

The **PREP-MB-DBS DWP DNA Extraction Kit** is intended for human genomic DNA extraction from dried blood spots (DBS).

WARNING! Intravenous injections of heparin, infusions of parenteral nutrition are not allowed if done less than 6 hours before the test.

General recommendations

The blood is applied to the sampling paper in an amount sufficient to obtain DBS with a diameter of at least 1 cm, and the blood must soak through the paper. After applying the sample, the filter card is dried horizontally on a clean, degreased surface for at least two hours without any external source of heat.

WARNING! It is not allowed to collect capillary blood into the tubes with coagulants, anticoagulants and fillers.

WARNING! Avoid direct sunlight (ultraviolet light), exposure to heat and moisture on the DBS samples during drying and storage!

Interfering substances

Hemoglobin (up to 250 mg/mL), bilirubin (up to 500 μmol/L), cholesterol (up to 12 mmol/L), triglycerides (up to 500 mg/L) do not inhibit PCR when extracting DNA from dried blood spots.

Components of the filter paper marking of the test form (if available on the filter card), wash solution No. 2 (up to 12.5%), and cresol red (up to 0.0125 ng/mL), which may get into the DNA sample during the extraction procedure, do not inhibit PCR.

Transportation and storage of samples

To prevent contamination and cross-contamination, filter cards with blood spots shall be packed individually after drying.

Transportation and storage of DBS samples on filter cards shall be carried out according to the manufacturer's instruction.

Sample preparation

7.1. Preparation for DNA extraction

7.1.1. Mark an empty high profile 96-well deep-well plate for DBS samples (is not included in the reagent kit).

7.1.2. Obtain three 3.0 – 3.2 mm discs from a patient's DBS into the plate well using a special automatic or hand puncher. Repeat the procedure for each sample.

7.1.3. Fill the corresponding plate wells with DBS discs. Do not introduce discs into the wells intended for negative control (C-).

WARNING! Fill the plate with discs considering the number of controls in the PCR assay (e.g. if two positive

controls and one negative control are included in the PCR kit, fill 93 out of 96 wells). An empty well not containing biomaterial sample (DBS discs) is used as a negative control (C-).
WARNING! The obtained disc must be soaked in blood. It is recommended to punch out the disc from the central part of the spot, without capturing the area of the borderline paint.

WARNING! When using a hand puncher, to reduce the risk of cross-contamination it is recommended to make 2–3 cuts on a clean part of filter paper before obtaining discs from each new DBS sample.

8. PROCEDURE

WARNING!

1. Manipulations with DBS should be carried out in compliance with sanitary and epidemiological rules similarly to the samples containing blood and other biological fluids.
2. For sample preparation and extraction use DNAse and RNAse free filter tips.
3. When adding reagents to the well containing biological material, be careful not to touch the walls of the well. Change the tip if it touches the walls of the well.
4. Test samples and negative control (C-) must be treated by the same scheme according to this instruction.
5. The lysis solution and wash solution No. 1 can form precipitate if stored in refrigerator (from 2 °C to 8 °C). Dissolve it by placing the vial, or plate on the thermostat warmed to 65 °C and warm until full precipitate dissolution. Cool the solution to room temperature (from 18 °C to 25 °C) before work. Alternatively, the precipitate can be dissolved at room temperature during 12 hours.

8.1. Pretreatment of samples (DBS discs)

WARNING! For sample pretreatment it is allowed to use high profile deep-well plates compatible with Auto-Pure 96 Extraction System. Using low profile plates may lead to cross-contamination of samples and false assay results.

8.1.1. Add 300 µL of lysis solution into each plate well with DBS discs (see 7.1.1 – 7.1.3) including the negative control (C-) well.

N o t e – When working with PREP-MB-DBS DWP, package P (Set No. 2), remove protective seal from deep-well plate containing lysis solution.

8.1.2. Prepare the Auto-Pure 96 Extraction System to DBS samples pretreatment by installing the deep-well plate with DBS samples and deep-well plate with tip comb according to the Annex A and the Auto-Pure 96 user manual.

8.1.3. Perform pretreatment of DBS samples using Auto-Pure 96 Extraction System[1].

8.1.4. Wait until Auto-Pure 96 finishes operation. Take out the plate with pretreated samples from Auto-Pure 96. Do not take out the plate with tip comb (if it is necessary, please keep the plate for the next stage — DNA extraction (8.2, 8.3).

8.1.5. After taking out the plates with pretreated samples from Auto-Pure 96 wait until the plate cools down to room temperature.

8.1.6. Prepare and mark as “No. 1” an empty 96-well deep-well plate for transferring of pretreated samples.

N o t e :
An empty 96-well deep-well plate is not included in the **PREP-MB-DBS DWP**, package N.
An empty 96-well deep-well plate is included in the **PREP-MB-DBS DWP**, package P (Set No. 1) — 96-well deep-well plate for pretreated samples.

[1] - To obtain scenario for Auto-Pure 96 Extraction System, please contact our Customer Service by e-mail hotline@dna-technology.ru.

When working with **PREP-MB-DBS DWP**, package P (Set No. 2), the plate with lysis solution may be used as a plate for transferring pretreated samples.

8.1.7. Take approximately 250-270 µL from each well (including the well with negative control) without touching DBS discs with the tip and transfer it to the corresponding well of the plate No. 1. Use separate tip for each well.

8.2. DNA extraction from pretreated samples using PREP-MB-DBS DWP, package N

8.2.1 Mark 4 new 96 deep-well plates:

- No. 2 – for sorbent;
- No. 3 – for wash solution No. 1;
- No. 4 – for wash solution No. 2;
- No. 5 – for elution solution.

8.2.2 Resuspend the sorbent thoroughly on vortex and add 250 µL of sorbent into each plate No. 2 well.

WARNING! It is necessary to shake the vial with sorbent regularly on vortex to mix the magnetic beads when spreading the sorbent into the plate wells.

8.2.3 Add 300 µL of wash solution No. 1 into each plate No. 3 well.

8.2.4 Add 700 µL of wash solution No. 2 into each plate No. 4 well.

8.2.5 Add 70 µL of elution solution into each plate No. 5 well.

WARNING! Make sure that you added the reagents into all of the wells of corresponding plates.

8.2.6 Install the prepared deep-well plates Nos. 2–4 (with reagents), plate No. 1 (with samples) and deep-well plate with tip comb (see 8.1.4) according to Annex A.

8.2.7 Perform DNA extraction using Auto-Pure 96 Extraction System[2].

8.2.8 Wait for the Auto-Pure 96 extraction system to finish operation.

DNA preparation is ready for PCR (plate No. 5 with elution solution). When storing the plate with extracted DNA, use plate seal.

8.3. DNA extraction from pretreated samples using PREP-MB-DBS DWP, package P (Set No. 1, Set No. 2)

8.3.1 Perform pretreatment of DBS samples (8.1.1–8.1.7).

8.3.2 Spin deep-well plates with sorbent, wash solution No. 1, wash solution No. 2 and elution solution (hereinafter deep-well plates with reagents) at RCF(g) 150 for 30 seconds.

8.3.3 Remove protective seal from deep-well plates with reagents.

8.3.4 Install the prepared deep-well plates with reagents, deep-well plate No. 1 (with samples) and deep-well plate with tip comb (see 8.1.4) according to Annex A and Auto-Pure 96 user manual.

8.3.5 Perform DNA extraction using Auto-Pure 96 Extraction System.

8.3.6 Wait until the extraction scenario is complete.

DNA preparation is ready for PCR (plate No. 5 with elution solution). When storing the plate with extracted DNA use plate seal.

8.4. DNA preparation storage and use

8.4.1. DNA preparation can be stored at 2 °C – 8 °C for up to 7 days and at minus 18 °C – minus 22 °C for up to

[2] - To obtain scenario for Auto-Pure 96 Extraction System, please contact our Customer Service by e-mail hotline@dna-technology.ru.

30 days.

8.4.2. If DNA preparations were stored at minus 18 °C – minus 22 °C, thaw them at room temperature (18 °C – 25 °C) or at 2 °C – 8 °C.

WARNING! Only one freezing-thawing of DNA preparation is allowed.

WARNING! Prior to adding DNA preparation to the tubes with amplification mixture mix it by pipetting 3-5 times.

9. SPECIFICATIONS

a. Recommended amount of biomaterial for DNA extraction: three 3.0—3.2 mm DBS discs.

b. Functional characteristics of the kit:

- DNA samples purity (A260/280): 1.0 – 1.2;
- DNA concentration in the preparation: 0.5 – 1.3 ng/µL (corresponds to 35 - 90 ng of DNA in 70 µL of obtained preparation).

c. Effectiveness of the reagent kit

Number of trials (N) – 173.

Effectiveness of the reagent kit – 100% (Ps = 98.68%).

d. Within-batch and between-batch precision

Within-batch precision – 100% (90.97% - 100%).

Between-batch precision – 100% (90.97% - 100%).

10. TROUBLESHOOTING

Problem	Possible cause	Solution
Insufficient amount of DNA in results of PCR.	Incorrect DBS sample preparation or storage (preliminary blood sampling from a tube with heparin, not enough blood on the paper discs, storages DBS under UV light). Plates with reagents stored upside down (for plate format, package P). Incorrect operation of Extraction System (incorrect heating function, incorrect level of magnetic rods immersion, incompatible consumable).	Take new samples and repeat the assay. Centrifuge the plates before removing the aluminum foil. Check compatibility of deep-well plates with Extraction System. Carry out service of Extraction System.
Absence of a positive reaction in a known positive DBS sample during PCR.	Incorrect DBS sample preparation or storage (preliminary blood sampling from a tube with heparin, storages under UV light). Incorrect sample processing (no reagent in the well (for bottle format, package N), error in the positioning of the deep-well plates with reagents).	Take new samples and repeat the assay. Check the positioning of the deep-well plates with reagents into Extraction System (see Annex A), make sure that all wells of plates are filled.
The presence of a positive reaction in a known negative sample during PCR.	Contamination at the stage of DNA extraction.	Use high profile deep-well plates (2.2 mL) for sample pretreatment. Use filter tips, chemical and ultraviolet disinfection of all work surfaces, use separate sets equipment for each area. Dispose of the current batch. Perform decontamination procedures.

11. QUALITY CONTROL

“DNA-Technology Research&Production”, LLC declares that the abovementioned products meet the provision of the Regulation (EU) 2017/746 of the European parliament and of the Council of 5 April 2017. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

- observation of quality management in manufacturing of IVDR products;
- creation of values for customers;
- maintenance of the best service quality and customer management.

Contact our customer service with quality issues of **PREP-MB-DBS DWP DNA Extraction Kit**.

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12. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Consult instructions for use
	Temperature limit	NON STERILE	Non-sterile
Σ	Contains sufficient for<n>tests		Date of manufacture
	Use-by date	REF	Catalogue number
LOT	Batch code		Manufacturer
VER	Version		Keep away from sunlight
	Caution		Do not re-use
EC REP	Authorized representative in the European Community		

Annex A

Scheme of PREP-MB-DWP deep-well plates placement

on the Auto-Pure 96 automatic extraction system heating plate:

Pretreatment stage:

PREP-MB-DBS DWP kit			**Auto-Pure 96 extraction system**	
Plate marking	Plate contents	Volume in the well	Plate position No.	Thermal regulation
No marking	No reagents, with tip comb	-	**1**	-
Custom marking	DBS samples\lysis solution	300 µL	**2**	**yes**

DNA extraction stage

PREP-MB-DBS DWP kit			**Auto-Pure 96 extraction system**	
Plate No.	Plate contents	Volume in the well	Plate position No.	Thermal regulation
No marking	No reagent, magnetic rods tip placed	-	**1**	-
Plate No. 1	DBS samples\ Lysis solution	300 µL	**2**	**yes**
Plate No. 2	Sorbent	250 µL	**3**	-
Plate No. 3	Wash solution No. 1	300 µL	**4**	-
Plate No. 4	Wash solution No. 2	700 µL	**5**	-
-	-	-	6	-
-	-	-	7	-
Plate No. 5	Elution solution	70 µL	**8**	**yes**

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