817-1 2022-07-25

For professional use only

PREP-CITO DBS DNA Extraction Kit

INSTRUCTION FOR USE

OBELIS S.A

Registered Address:

Bd. Général Wahis, 53

1030 Brussels, Belgium

Tel: +32.2.732.59.54

Fax: +32.2.732.60.03

E-mail: mail@obelis.net

http://www.obelis.net

"DNA-Technology Research & Production", LLC,

142281, Russia,

Moscow Region, Protvino,

Zheleznodorozhnaya Street, 20

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

Customer service department

E-mail: hotline@dna-technology.ru

REF P-029-N/2EU

VER 817-1.2022.07.25

TABLE OF CONTENTS

1. INTENDED USE

The **PREP-CITO DBS DNA Extraction Kit** is intended for human genomic DNA extraction from dried blood spots (DBS) for further analysis with polymerase chain reaction (PCR).

The **PREP-CITO DBS DNA Extraction Kit** is intended for *in vitro* diagnosis.

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

The **PREP-CITO DBS 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 kits only as directed in this instruction for use.

2. METHOD

The **PREP-CITO DBS DNA Extraction Kit** is based on alkaline lysis. The principle of the method is alkaline cell lysis, which occurs during thermal incubation. The removal of possible interfering substances from the carrier takes place at the pre-washing stage.

3. CONTENT

The detailed description of content is represented in Table 1.

Table 1. The **PREP-CITO DBS DNA Extraction Kit** content for P-029-N/2EU, package N

Reagent	Description	Total volume	Amount
Lysis solution	Colorless transparent liquid	20 mL	1 vial
Neutralizing solution	Colorless transparent liquid	400 µL	1 tube
Wash solution H	Colorless transparent liquid	70 mL (35 mL in each vial)	2 vials
Wash solution CK	Colorless transparent foamy liquid	35 mL	1 vial

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

The **PREP-CITO DBS DNA Extraction Kit** is designed for DNA extraction from 50 analyzed samples (including negative controls).

4. REAGENTS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

4.2 Specimen collection

- For blood collection: 2.0 - 4.0 mL Vacuette blood collection tubes with anticoagulant, for example, salt of EDTA at a final concentration of 2.0 mg/mL or sodium citrate anticoagulant.

Please use only salt of EDTA or sodium citrate as an anticoagulant, since other substances can provide PCR inhibition.

4.3 DNA extraction

- Biological safety cabinet class II;
- Refrigerator;
- Vortex mixer;
- High speed centrifuge (RCF(g) 16000);
- Solid-state thermostat (temperature range 25-90 °C) (for example, TT-1 or TT-2, made by "DNA-Technology");
- Tube rack for 1.5 mL tubes;
- DNase and RNase free 1.5 mL tubes;
- Electric laboratory aspirator with trap flask for the removal of supernatant;
- Single channel pipettes (dispensers covering 2.0-1000 µL volume range);
- RNase and DNase free filtered pipette tips (volume 20 µL, 200 µL, 1000 µL);
- RNase and DNase free pipette tips for aspirator with trap flask;
- Perforator for obtaining paper discs 3.0-3.2 mm in diameter from dried blood samples;
- Container for used pipette tips, tubes and other consumables;
- Powder-free surgical gloves;
- Disinfectant solution.

5. STORAGE AND HANDLING REQUIREMENTS

Expiry date – 12 months from the date of production.

All components of the **PREP-CITO DBS DNA Extraction Kit** must be stored at temperatures from 2 °C to 8 °C during the storage period.

To prevent contamination and cross-contamination, test forms with blood stains are individually packed after drying. Storage of test forms with dry blood samples up to one year is acceptable in conditions of low humidity (not more than 30%) at a temperature of 2 °C to 8 °C. For longer periods of storage, dry blood samples should be stored as recommended by the manufacturer of the test forms.

The kit has to be transported in thermoboxes with ice packs by all types of roofed transport at temperatures corresponding to storage conditions of the kit components.

It is allowed to transport the kit in thermobox with ice packs by all types of roofed transport at temperatures from 2 °C to 25 °C but no more than 7 days and should be stored at temperatures from 2 °C to-8 °C immediately on receipt.

Shelf-life of the kit following the first opening of the primary container: the components of the kit should be stored at temperatures of 2 °C to 8 °C during the storage period.

The kit stored in under undue regime should not be used.

An expired **PREP-CITO DBS DNA Extraction Kit** should not be used.

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

The conformity of the **PREP-CITO DBS DNA Extraction Kit** to the prescribed technical requirements is subject to compliance of storage, transportation and handling conditions recommended by manufacturer.

Contact our official representative in EU by quality issues of the **PREP-CITO DBS DNA Extraction Kit**.

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, 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-CITO DBS DNA Extraction Kit** is intended for human genomic DNA extraction from dried blood spots.

General recommendations

Preparation of blood spots, transport and storage dry blood spots should be done in accordance with the manufacturer's instructions to the sampling filter.

DNA extraction can be performed from DBS obtained from native capillary blood or venous blood.

Interfering substances

DNA extraction can be performed from venous or capillary blood spots applied to a test form made of Whatman 903 or similar filter paper.

Cellulose fiber and residual amounts of chemical components of the infiltration and labeling of the recommended filter paper remaining in the DNA sample as a result of incomplete removal during sample preparation do not affect the PCR results.

Blood sampling

Blood sampling is carried out in vacuum plastic tube. It may be 2.0 or 4.0 mL Vacuette blood collection tubes with anticoagulant, for example salt of EDTA at a final concentration of 2.0 mg/mL or sodium citrate anticoagulant. After taking the material, it is necessary to mix the blood with anticoagulant turning the tube 8 – 10 times.

ATTENTION! It is not allowed to use heparin as an anticoagulant.

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

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 penetrate the paper. After applying the sample, the filter card is dried horizontally on a clean, degreased surface for at least two hours at room temperature (18 °C to 25 °C) without any external source of heat and direct sunlight.

To carry out the DBS test, 3.0 mm discs from the DBS shall be obtained using a hand puncher or similar equipment, according to the manufacturer's instructions.

Sample preparation

Preparation for DNA extraction from dried blood spots:

1 Mark the required number of 1.5 mL tubes considering the number of samples to be tested and negative control (C-).

2 Obtain a paper disc from a dried blood spot using a puncher. Three 3.0 mm discs must be obtained of the filter card to obtain one DNA preparation (three 3.2 mm discs are acceptable).

ATTENTION! The discs must be completely saturated with blood. It is recommended to obtain the discs out of the central area of the spot, without taking up the area of the bounding part.

ATTENTION! Before obtaining each new DBS sample it is recommended to punch out 2-3 discs from a clean sampling paper to avoid the risk of contamination of a new sample with the fragments of previous DBS sample.

3 Place the punched discs (3 discs for each sample) into the marked tubes for the samples to be tested, close the caps.

ATTENTION! It is recommended to punch discs from DBS immediately before DNA extraction procedure.

4 After completing the procedure sanitize the punch blade according to the instructions.

Transportation and storage of the samples

To prevent contamination and cross-contamination, filter cards with blood spots shall be individually packed after drying. The storage of samples with dry blood specimens for up to one year is acceptable in low humidity conditions (max. 30%) at a temperature from 2 °C to 8 °C. For longer storage periods, dry blood spot specimens should be stored as recommended by the manufacturer.

Dried blood samples may be transported in individual packages in conditions of low humidity (not more than 30%) at up to 25 °C for no more than 5 days.

8. PROCEDURE

ATTENTION!

1 DBS should be handled according to all the epidemiological rules and in the same manner as samples containing blood and other biological liquids.

2 Use disposable RNase- and DNase-free filter tips during the preparation and DNA extraction steps of the biomaterial.

3 Remove supernatant from each tube with a separate tip.

4 When adding reagents to the sample containing biological material, carefully add the solution without touching the walls of the tubes. If you touch the wall of the tube, change the tip.

5 To prevent contamination, open only the cap of the tube to be manipulated and close it before working with the next tube.

6 The samples to be tested and the negative control sample (C-) must be handled according to the same scheme according to these instructions.

8.1. DNA extraction

8.1.1. Add 1400 µL each of Wash solution H to all marked tubes with discs and to the negative control sample (C-) tube.

8.1.2. Mix the tubes content thoroughly for 10-20 seconds on a vortex mixer.

ATTENTION! The discs should stay in the solution and not stick together.

8.1.3. Place the tubes horizontally and incubate at room temperature (18 °C to 25 °C) for 5 minutes. During this incubation period the tubes content should be mixed 2-3 times within 10-20 seconds.

8.1.4. Centrifuge the tubes at RCF(g) 16000 for 5 minutes.

8.1.5. Draw off the supernatant solution without placing the tip on the discs and leave 5.0 – 10.0 µL of solution in the tube. Do not remove the discs from the tube.

8.1.6. Add 500 µL of Wash solution CK to all tubes.

8.1.7. Mix the tubes content thoroughly for 10-20 seconds on a vortex mixer.

8.1.8. Place the tubes horizontally and incubate at room temperature (18 °C to 25 °C) for 1-2 minutes. During this incubation period the tubes content should be mixed 2-3 times within 10-20 seconds.

8.1.9. Centrifuge the tubes at RCF(g) 16000 for 5 minutes.

8.1.10. Remove the supernatant completely, without placing the tip on the discs. Do not remove the discs from the tube.

8.1.11. Add 100 µL each of the Lysis solution to all tubes.

8.1.12. Mix the tubes content thoroughly for 10-20 seconds on a vortex mixer.

ATTENTION! The discs should be rinsed with the Lysis solution and not stick together.

8.1.13. Precipitate the drops from the caps of the tubes for 5-10 seconds on a vortex mixer.

8.1.14. Place the tubes in a thermostat preheated to 90 °C for 20 minutes.

8.1.15. Use an active final tube cooling program for the thermostat or carefully remove the tubes from the thermostat and allow them to cool vertically at room temperature (18 °C to 25 °C).

8.1.16. Centrifuge the tubes at RCF(g) 16000 for 30 seconds to precipitate condensate from the caps.

8.1.17. Mix the tube with Neutralizing solution on vortex mixer for 1-3 seconds. Spin down the drops from caps in vortex mixer for 1-3 seconds.

8.1.18. Add 2 µL of the Neutralizing solution to each tube.

8.1.19. Mix the tubes content thoroughly on a vortex mixer for 10 seconds.

8.1.20. Centrifuge the tubes at RCF(g) 16000 for 30 seconds.

The DNA sample is ready to use.

The DNA sample should be stored at 2 °C to 8 °C for up to 30 days and at minus 18 °C to minus 22 °C for up to 6 months (If recommendations for sample storage are not specified by PCR kit manufacturer).

If DNA products were stored at a temperature from minus 18 °C to minus 22 °C, they should be thawed at room temperature (from 18 °C to 25 °C) or at 2 °C to 8 °C.

ATTENTION! It is only allowed to thaw the DNA preparation once!

8.2. Modification of DNA extraction

If the recommendations for blood collection, transportation and storage of dried blood spots, technological requirements during DNA extraction are not followed, the efficiency of PCR may be reduced or PCR may be inhibited. In this case it is recommended to repeat the DNA extraction.

8.2.1. Mark one 1.5 mL disposable tube for each sample and for the negative control sample (C-).

8.2.2. Obtain a paper disc with the dried blood spot using a puncher. Three 3.0 mm diameter discs must be made out of the filter card to obtain one DNA preparation (three 3.2 mm diameter disks are acceptable). Place the discs (3 for each sample) into the marked tubes and close the lids.

8.2.3. Add 1400 µL of Wash solution H to each tube.

8.2.4. Mix the tubes content thoroughly for 10-20 seconds on a vortex mixer.

ATTENTION! The discs should stay in the liquid and not stick together.

8.2.5. Place the tubes horizontally and incubate at room temperature (18 °C to 25 °C) for 10 minutes. During the incubation time mix the tubes 2-3 times for 10-20 seconds on a vortex mixer.

8.2.6. Centrifuge the tubes at RCF(g) 16000 for 5 minutes.

8.2.7. Remove the supernatant without placing the tip on the discs and leaving 5.0 – 10.0 µL of liquid in the tube. Do not remove the discs from the tube.

8.2.8. Add 1000 µL of Wash solution CK to all tubes.

8.2.9. Repeat the actions from paragraphs 8.1.7 – 8.1.20.

ATTENTION! In case of serious faults at preanalytical stage, another sampling for further DBS production and/or DNA extraction from venous blood may be necessary.

9. SPECIFICATIONS

Effectiveness characteristics

The amount of DNA in 100 µl of the preparation extracted from three dried blood spot paper discs with a diameter of 3.0 mm: 30 - 140 ng. (The amount of extracted DNA depends on the number of white blood cells in the sample).

DNA yield (efficiency) ranges from 41.1 ng to 54.6 ng when extracted from 10 µL of blood dried on three filter card disks with varying white blood cell counts from 5.5 x 10^9/L to 7.8 x 10^9/L.

10. QUALITY CONTROL

"DNA-Technology Research&Production", LLC declares that the abovementioned products meet the provision of the Council Directive 98/79/EC for *in vitro* Diagnostic Medical Devices. The quality control procedures performed in accordance with ISO 9001:2015 and ISO 13485:2016:

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

Contact our official representative in EU by quality issues of the **PREP-CITO DBS DNA Extraction Kit**.

Technical support:

E-mail: hotline@dna-technology.ru

https://www.dna-technology.com

Manufacturer: "DNA-Technology Research & Production", LLC,

142281, Russia, Moscow Region,

Protvino, Zheleznodorozhnaya Street, 20

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

Seller: "DNA-Technology" LLC,

117587, Russia, Moscow,

int. ter. Municipal District Chertanovo Severnoye,

Varshavskoye shosse, 125 Zh, building 5, floor 1, office 12;

Phone/fax: +7(495) 640.17.71

E-mail: info@dna-technology.com

https://www.dna-technology.com

Authorized representative in EU:

OBELIS S.A

Registered Address:

Bd. Général Wahis, 53

1030 Brussels, Belgium

Tel: +32.2.732.59.54

Fax: +32.2.732.60.03

E-mail: mail@obelis.net

http://www.obelis.net

11. KEY TO SYMBOLS

IVD	*In vitro* diagnostic medical device		Manufacturer
	Temperature limit		Date of manufacture
Σ	Contains sufficient for <n> tests		Consult instructions for use
	Use-by date	REF	Catalogue number
LOT	Batch code	VER	Version
EC REP	Authorized representative in the European Community		Caution

REF P-029-N/2EU

VER 817-1.2022.07.25