

MB583PC16200

HiPurA® Pre- filled Clinical Multi- purpose Nucleic Acid Purification Kit (Cartridges)

Kit Contents

Product Code	Reagents provided	MB583PC16200
		48 PR
PF16E	Pre-filled Cartridges for Clinical Multi- purpose Purification	48 no
LA1118B	Magnetic Rod Tip	12 no
DS0015	Lysis Solution (AL)	9 ml
DS0013	Proteinase K	1 ml
DS0003	RNase A (20mg/ml)	1 ml
DS1005A	Magnetic Beads	1 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	4.5 ml

Intended Use

Recommended for isolation of nucleic acids from various samples like blood, plasma, serum, saliva, buccal swabs, buffy coat, cells, urine and tissue, nasopharyngeal swab, oropharyngeal swab in Viral Transport Medium.

Introduction

HiPurA® Pre- filled Clinical Multi- purpose Nucleic Acid Purification Kit (Cartridges) for Insta NX® Mag16 provides the fastest and easiest way to purify nucleic acid for reliable use in amplification technologies. HiPurA® Pre- filled Clinical Multi- purpose Nucleic Acid Purification Kit (Cartridges) can be used for isolation of nucleic acid from a wide variety of samples, but the performance may vary depending on the sample type.

HiPurA® Pre- filled Clinical Multi- purpose Nucleic Acid Purification Kit (Cartridges)

This kit carries out efficient extraction of genomic nucleic acid from wide range of samples. Sample is first lysed under the highly denaturing conditions provided by Lysis Solution to inactivate DNases/ RNases and to ensure isolation of intact genomic nucleic Acid. The purification procedure comprises of three steps viz. adsorption of nucleic acid to the magnetic beads, removal of residual contaminants and elution of pure nucleic acid. The magnetic beads have a high binding capacity and high-quality nucleic acid is obtained. The nucleic acid obtained is compatible with downstream applications such as restriction enzyme digestion, PCR and Southern blotting.

Elution

The yield of nucleic acid depends on the sample type and the number of cells in the sample. A single elution with the Elution Solution will provide sufficient nucleic acid to carry out multiple amplification reactions.

Storage

HiPurA® Pre- filled Clinical Multi- purpose Nucleic Acid Purification Kit (Cartridges) can be stored at room temperature (15-25°C) for up to 2 years without showing any reduction in performance. We advise a certain storage temperature for the reagents listed below:

- **On receipt store RNase A (DS0003): at 2-8°C**
- **On receipt store Proteinase K (DS0013): at -20°C**
- **On receipt store Magnetic Beads (DS1005A): at 2-8°C**

Materials needed but not provided

- 55°C heating block (For Tissue Preparation) [Wee Dry-® LA1115]
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes) [TabSpin- LA1090]
- Centrifuge with 15ml rotor
- 1X PBS (Product Code: ML116)
- Insta NX® Mag16 (Product Code: LA1118)
- Insta NX® Mag32 (LA1096)
- Cartridge Holder (Product Code: LA1118CH)
- Vortex
- Polypropylene sealing film (Product Code: PR21)
- Centrifuge Tube, 15ml (Product Code: PW1306)
- Sterile foam Tipped Swab (Product Code: PW1174)
- Trypsin (Product Code: TC598)
- 0.5M EDTA (pH 8.0) (For Urine Preparation) (Product Code: ML014)
- HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017)

General Preparation Instructions

1. Preheat a water bath or heating block to 55°C.
(For Tissue Preparation)

2. **Thoroughly mix reagents**

Examine the reagents for precipitation. If any kit reagent forms a precipitate (other than enzymes), warm at 55-65°C until the precipitate dissolves and allow cooling to room temperature (15-25°C) before use.

3. Ensure that clean & dry Nuclease-free tubes and tips are used for the procedure.
4. Vortex magnetic beads before use.

RNase A enzyme treatment

RNase A is a type of RNase that is commonly used in research. RNase A (e.g. bovine pancreatic ribonuclease A) is one of the sturdiest enzymes in common laboratory usage. It cleaves 3' end of unpaired C and U residues.

Unit Definition for RNase A

One unit of the enzyme causes an increase in absorbance of 1.0 at 260 nm when yeast RNA is hydrolyzed at 37°C and pH 5.0. Fifty units are approximately equivalent to 1 Kunitz unit. It is completely free of DNases. The specific activity is 90 U/mg.

The product as supplied is stable at room temperature (15-25°C).

Specimen Handling and Collection

Collect cells, tissues, blood sample, serum, plasma in a sterile container and freeze the sample at -20°C for short term storage or -80°C for long term storage. Collect whole blood in an anticoagulant tube (an EDTA tube is preferred) under sterile conditions (if to be used for future) and store the samples at 2-8°C for short term storage or -20°C for long term storage.

Incubate frozen cells, tissues, blood sample, serum, plasma samples on ice for complete thawing. Ensure that the samples are at room temperature (15-25°C) before beginning the protocol.

Collect the buccal/gargled sample with the help of a swab and store the samples at 2-8°C for short term storage or -20°C for long term storage. Incubate the frozen samples on ice for complete thawing. Ensure that the samples are at room temperature (15-25°C) before beginning the protocol.

Collect stool/ Urine sample in a sterile container (if to be used for future) and store the samples at 2-8°C for short term storage or -20°C for long term storage. Ensure that the sample is at room temperature (15-25°C) before beginning the protocol.

After use, contaminated material must be sterilized by autoclaving before discarding.

Type of Specimens

Clinical samples: tissue, blood, cells, serum, plasma, saliva, buccal swabs, buffy coat, urine, nasopharyngeal swab, oropharyngeal swab in Viral Transport Medium

Tissue/ Cells

Tissue Preparation

I. Prepare tissue

Weigh a piece of fresh or frozen tissue and mince quickly. If frozen tissue is used, allow it to thaw slightly before slicing but keep on ice in order to protect degradation. Cut the tissue into small pieces as it enables more efficient lysis. Up to 25 mg of tissue (or 10 mg of spleen, due to the high number of cells per given mass) may be used per preparation. Transfer to a capped 2.0 ml collection tube (not provided) and continue to step II of Tissue Preparation.

NOTE: Tissue can be harvested, by aliquoting in 2.0 ml collection tubes (not provided) and flash freezing in liquid nitrogen; these can be stored at -70°C for several months before preparing nucleic acid.

II. Digest tissue

Add 180 µl of Lysis Solution (AL) (DS0015) and 20 µl of the Proteinase K solution to the tissue. Mix by vortexing. Incubate the sample at 55°C until the tissue is completely digested with no particles remaining. Mix by vortexing occasionally or use a shaking water bath. Digestion is usually complete in 2 to 4 hours. Vortex briefly after digestion is completed. **This will be your pre- processed sample.**

Cultured Cell Preparation

I. Harvest cells

- **Attached cell cultures:** The cells can be detached using trypsin. Centrifuge upto 5×10^6 cells for 5 minutes at 300 x g (≈ 1500 rpm). Discard the culture medium and continue with step II of Cultured Cell Preparation.

- **Suspension cell cultures:** Centrifuge upto 5×10^6 cells for 5 minutes at $300 \times g$ [≈ 1500 rpm]. Discard the culture medium completely and continue with step II of Cultured Cell Preparation.
- II. Resuspend the pellet obtained from step I of Cultured Cell Preparation, in capped 2ml centrifuge tube (not provided) add 200 μ l of Resuspension Solution (1X PBS) (ML116) (not provided) and mix thoroughly. If previously frozen, allow the cell pellet to thaw slightly before resuspending. Add 20 μ l Proteinase K (DS0013). **This will be your pre- processed sample.**

Set up processing cartridge as follows:

1. Switch on the UV light for 10 minutes prior to use.
2. Select “**M583TUCS**” program.
3. Open the door of Insta NX[®] Mag16 machine.
4. Place the Pre- filled cartridges for Clinical Multi- purpose Purification (PF16E) into the Cartridge Holder (LA1118CH). Remove the seal from the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).

NOTE: Take care while peeling off the seal. Hold the cartridge firmly by one hand and then peel off the seal by holding it in your other hand without shaking the cartridge.

5. Add 50 μ l of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040) into the 6th well of the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).
6. Add 200 μ l pre- processed sample in the 1st well of the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).

Optional RNase A treatment

If RNA-free genomic DNA is required, add 20 μ l of RNase A solution (20 mg/ml) (DS0003) in the 1st well of the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).

7. **Add 20 μ l Magnetic Beads (DS1005A) in the 1st well of the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).**
8. After adding the above solutions place the cartridges along with the cartridge holder on the platform.
9. Place the Magnetic Rods Tip (LA1118B) by sliding onto the machine.

NOTE: After placing the rods ensure that the rods are properly fixed on their place.

10. Click on the **RUN** option on the home screen.
11. After the run is complete, remove Cartridge Holder (LA1118CH) & cartridge from the position. Discard the Magnetic Rods Tip (LA1118B). Dispense the eluted nucleic acid from well 6 to a new HiPer[®] Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017) (not provided). The eluate contains pure nucleic acid.

NOTE: If small amount of magnetic beads are observed in the final eluate then keep the cartridges along with cartridge holder on Magnetic pad (not provided) for 4-5 minute and collect supernatant carefully without disturbing beads pellet in new collection tube.

OR

Take out eluate in new collection tube and centrifuge at higher speed for around 1 min to pellet down the traces of Magnetic beads present in the eluate.

Saliva/ Buccal Swab

Buccal Swab Preparation

NOTE: We recommend using Sterile foam Tipped Swab (Product Code: PW1174) (not provided) for collection of sample from inside of cheek to ensure maximum yield

1. Place the buccal swab / gargled sample into a capped 2.0 ml microcentrifuge tube (not provided). Add 400 µl of 1X PBS (ML116) (not provided) to the tube.
2. Centrifuge the tube at 13,000 rpm for 2 minutes. Discard the pellet and transfer the supernatant to a new collection tube (not provided). **This will be your pre- processed sample.**

Set up processing cartridge as follows:

1. Switch on the UV light for 10 minutes prior to use.
2. Select “**M583SBLs**” program.
3. Open the door of Insta NX® Mag16 machine.
4. Place the Pre- filled cartridges for Clinical Multi- purpose Purification (PF16E) into the Cartridge Holder (LA1118CH). Remove the seal from the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).

NOTE: Take care while peeling off the seal. Hold the cartridge firmly by one hand and then peel off the seal by holding it in your other hand without shaking the cartridge.

5. Add **50µl of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040)** into the **6th well of the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).**
6. Add **20µl of Proteinase K (DS0013) and 400µl saliva/pre- processed sample** in the **1st well of the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).**

Optional RNase A treatment

If RNA-free genomic DNA is required, add **20 µl of RNase A solution (20 mg/ml) (DS0003)** in the **1st well of the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).**

7. **Add 20µl Magnetic Beads (DS1005A) in the 1st well of the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).**
8. After adding the above solutions place the cartridges along with the cartridge holder on the platform.
9. Place the Magnetic Rods Tip (LA1118B) by sliding onto the machine.

NOTE: After placing the rods ensure that the rods are properly fixed on their place.

10. Click on the **RUN** option on the home screen.
11. After the run is complete, remove Cartridge Holder (LA1118CH) & cartridge from the position. Discard the Magnetic Rods Tip (LA1118B). Dispense the eluted nucleic acid from well 6 to a new HiPer® Lock

Microcentrifuge Tube, 2.0ml (Product Code: MBLA017) (not provided). The eluate contains pure nucleic acid.

NOTE: If small amount of magnetic beads are observed in the final eluate then keep the cartridges along with cartridge holder on Magnetic pad (not provided) for 4-5 minute and collect supernatant carefully without disturbing beads pellet in new collection tube.

OR

Take out eluate in new collection tube and centrifuge at higher speed for around 1 min to pellet down the traces of Magnetic beads present in the eluate.

Blood/ Serum/ Plasma/ Buffy coat/ Nasopharyngeal/ Oropharyngeal swab

Set up processing cartridge as follows:

1. Switch on the UV light 10 mins prior to use.
2. Select “**M583BPSS**” program.
3. Open the door of Insta NX[®] Mag16 machine.
4. Place the Pre- filled cartridges for Clinical Multi- purpose Purification (PF16E) into the Cartridge Holder (LA1118CH). Remove the seal from the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).
5. **NOTE: Take care while peeling off the seal. Hold the cartridge firmly by one hand and then peel off the seal by holding it in your other hand without shaking the cartridge**
6. Add 50µl of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040) into the 6th well of the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).
7. Add 20µl of Proteinase K (DS0013) and 200µl of sample in the 1st well of the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).

Optional RNase A treatment

If RNA-free genomic DNA is required, add 20 µl of RNase A solution (20 mg/ml) (DS0003) in 1st well of the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).

8. **Add 20µl Magnetic Beads (DS1005A) in the 1st well of the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).**
9. After adding the above solutions place the cartridges along with the cartridge holder on the platform.
10. Place the Magnetic Rods Tip (LA1118B) by sliding onto the machine.

NOTE: After placing the rods ensure that the rods are properly fixed on their place.

11. Click on the **RUN** option on the home screen.
12. After the run is complete, remove Cartridge Holder (LA1118CH) & cartridge from the position. Discard the Magnetic Rods Tip (LA1118B). Dispense the eluted nucleic acid from well 6 to a new HiPer[®] Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017) (not provided). The eluate contains pure nucleic acid.

NOTE: If small amount of magnetic beads are observed in the final eluate then keep the cartridges along with cartridge holder on Magnetic pad (not provided) for 4-5 minute and collect supernatant carefully without disturbing beads pellet in new collection tube.

OR

Take out eluate in new collection tube and centrifuge at higher speed for around 1 min to pellet down the traces of Magnetic beads present in the eluate.

Urine

Sample Pre-treatment procedure

- Centrifuge 5 ml of urine sample in 15 ml centrifuge tube (not provided) at 13,000 rpm for 5 min. Resuspend the pellet in 500 µl of 1X PBS (not provided).
- Add 30 µl of 0.5M EDTA (pH 8.0) (not provided). Mix and centrifuge at 13,000 rpm for 2 min.
- Resuspend the pellet with 200 µl of 1X PBS (not provided) and mix well. **This will be your pre-processed sample.**

Set up processing cartridge as follows:

1. Switch on the UV light for 10 minutes prior to use.
2. Select “**M583TUCS**” program.
3. Open the door of Insta NX® Mag16 machine.
4. Place the Pre- filled cartridges for Clinical Multi- purpose Purification (PF16E) into the Cartridge Holder (LA1118CH). Remove the seal from the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).

NOTE: Take care while peeling off the seal. Hold the cartridge firmly by one hand and then peel off the seal by holding it in your other hand without shaking the cartridge.

5. Add **50µl of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040)** into the **6th well of the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).**
6. Add **20µl of Proteinase K (DS0013) and 200µl pre- processed sample** in the **1st well of the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).**

Optional RNase A treatment

If RNA-free genomic DNA is required, add **20 µl of RNase A solution (20 mg/ml) (DS0003)** in the **1st well of the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).**

7. **Add 20µl Magnetic Beads (DS1005A) in the 1st well of the Pre-filled Cartridges for Clinical Multi- purpose Purification (PF16E).**
8. After adding the above solutions place the cartridges along with the cartridge holder on the platform.
9. Place the Magnetic Rod Tip (LA1118B) by sliding onto the machine.

NOTE: After placing the rods ensure that the rods are properly fixed on their place.

10. Click on the **RUN** option on the home screen.

11. After the run is complete, remove Cartridge Holder (LA1118CH) & cartridge from the position. Discard the Magnetic Rods Tip (LA1118B). Dispense the eluted nucleic acid from well 6 to a new HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017) (not provided). The eluate contains pure nucleic acid.

NOTE: If small amount of magnetic beads are observed in the final eluate then keep the cartridges along with cartridge holder on Magnetic pad (not provided) for 4-5 minute and collect supernatant carefully without disturbing beads pellet in new collection tube.

OR

Take out eluate in new collection tube and centrifuge at higher speed for around 1 min to pellet down the traces of Magnetic beads present in the eluate.

***NOTE: If you process less than 4 samples at a time please order LA1118B- Magnetic Rod Tip for Insta NX® Mag16 (Pack size- LA1118B-4no/ LA1118B-40no).**

Storage of the eluate with purified nucleic acid: The eluate contains pure nucleic acid, recommended to be stored at lower temperature (-80°C). Avoid repeated freezing and thawing of the sample which may cause denaturing of nucleic acid.

Warning

Not for Medicinal Use. Read the procedure carefully before beginning the protocol. Wear protective gloves/protective clothing/eye protection/face protection. Follow good clinical laboratory practices while handling clinical samples. Standard precautions should be followed as per established guidelines. Safety guidelines may be referred in safety data sheets of the product.

Limitations

The yield of nucleic acid depends upon the type and the volume of starting material used.

Performance and Evaluation

The yield and efficiency of purification is determined by performing Real- Time PCR.

Quality Control

Each lot of HiMedia's HiPurA® Pre- filled Clinical Multi- purpose Nucleic Acid Purification Kit (Cartridges) is tested against predetermined specifications to ensure consistent product quality.

Safety Information

The HiPurA® Pre- filled Clinical Multi- purpose Nucleic Acid Purification Kit (Cartridges) is for laboratory use only, not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing bleach. Please refer the Safety Data Sheet (SDS) for information regarding hazards and safe handling practices.

Disposal










User must ensure safe disposal by autoclaving and/ or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed off in accordance with current laboratory techniques.

Please refer disclaimer Overleaf.

Technical Assistance

At HiMedia, we pride ourselves on the quality and availability of our technical support. For any kind of technical assistance mail to mb@himedialabs.com.

Symbols

	Manufacturer		Do not use if package is damaged
	Catalogue number		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Contains biological material of animal origin
	Batch code		

Identification No.: PIMB583PC16200

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Disclaimer :

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal or therapeutic use but for laboratory, diagnostic, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

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