# MB583MPF16200 HiPurA® Pre- filled Clinical Multi- purpose Nucleic Acid Purification Kit (Plates)

**Kit Contents**

| Product Code | Reagents provided | MB583MPF16200 |
|---|---|---|
| | | 96PR |
| PF16F | Pre-filled Plates for Clinical Multi- purpose Purification | 6 no |
| LA1118B | Magnetic Rod Tip | 12 no |
| DS0015 | Lysis Solution (AL) | 18 ml |
| DS0013 | Proteinase K | 2 ml |
| DS0040 | Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5] | 7 ml |
| DS0003 | RNase A (20mg/ml) | 2 ml |
| DS1005A | Magnetic Beads | 2 ml |

**Intended Use**

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

**Introduction**

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

This kit carries out efficient extraction of 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 nucleic acid. The nucleic acid 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 from various species. 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 Elution Solution will provide sufficient nucleic acid to carry out multiple amplification reactions.



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Registered Office
HiMedia Laboratories Pvt Ltd.
Plot No. C-40, Road No. 21Y, MIDC, Wagle Industrial Estate,
Thane, (West) 400604, Maharashtra, INDIA.
Customer Care No.: 00-91-22-6116 9797
Tel : 00-91-22-6147 1919, 6903 4800

Fax : 6147 1920
Web : www.himedialabs.com
Email : info@himedialabs.com
mb@himedialabs.com

**Storage**

HiPurA® Pre- filled Clinical Multi- purpose Nucleic Acid Purification Kit (Plates) 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)
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Centrifuge with 15ml rotor
- Centrifuge Tube, 15ml (Product Code: PW1306)
- 1X PBS (Product Code: ML116)
- Insta NX® Mag16 (Product Code: LA1118)
- Vortex
- Polypropylene sealing film (Product Code: PR21)
- HiPer® Lock Microcentrifuge Tube, 2.0ml (Product Code: MBLA017)
- Sterile foam Tipped Swab (Product Code: PW1174)
- Trypsin (Product Code: TC598)
- 0.5M EDTA (pH 8.0) (For Urine Preparation)

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

Collect 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

**Procedure**

## 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 56°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 up to 5 x $10^6$ 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 up to 5 x $10^6$ cells for 5 minutes at 300 x 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 µl 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 µl Proteinase K (DS0013). **This will be your pre- processed sample.**

### Set up processing plates as follows:

1. Switch on the UV light for 10 minutes prior to use.
2. Open the door of Insta NX® Mag16 machine.
3. Select **"M583TUCS"** program.
4. Remove the seal from the Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).

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

5. Add **50µl of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040)** into the **6th and 12th column of the Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).**
6. **Add 180-200µl pre- processed sample in the 1st and the 7th column of the Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).**

   **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 and the 7th column of the Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).

7. **Add 20µl Magnetic Beads (DS1005A) in the 1st and the 7th column of the Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).**

   **NOTE: 16 samples can be processed in a single Pre-filled Plate for Clinical Multi- purpose Purification (PF16F)**

8. After adding the above solutions place the plates on the tray of the machine.
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 the 96 Deep-well Plate from the position. Discard the Magnetic Rod Tip (LA1118B). Dispense the eluted nucleic acid from column 6 and column 12 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. Add 400 µl of 1X PBS (ML116) 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 plates as follows:

1. Switch on the UV light for 10 minutes prior to use.
2. Open the door of Insta NX® Mag16 machine.
3. Select **"M583SBLS"** program.
4. Remove the seal from the Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).

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

5. Add **50µl of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040)** into the **6th and 12th column of the Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).**
6. **Add 20µl of Proteinase K (DS0013) and 500µl saliva/pre- processed sample in the 1st and the 7th column of the Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).**

   **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 and the 7th column of the Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).

7. **Add 20µl Magnetic Beads (DS1005A) in the 1st and the 7th column of the Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).**

   **NOTE: 16 samples can be processed in a single Pre-filled Plates for Clinical Multi- purpose Purification (PF16F)**

8. After adding the above solutions place the plates on the tray of the machine.

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 the 96 Deep-well Plate from the position. Discard the Magnetic Rod Tip (LA1118B). Dispense the eluted nucleic acid from column 6 and column 12 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

1. Switch on the UV light for 10 minutes prior to use.
2. Open the door of Insta NX® Mag16 machine.
3. Select **"M583BPSS"** program.
4. Remove the seal from the **Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).**

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

5. Add **50µl of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040)** into the **6th and 12th column of the Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).**
6. **Add 20µl of Proteinase K (DS0013) and 200µl of sample in the 1st and the 7th column of the Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).**

   **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 and the 7th column of the Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).

7. **Add 20µl Magnetic Beads (DS1005A) in the 1st and the 7th column of the Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).**

   **NOTE: 16 samples can be processed in a single Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).**

8. After adding the above solutions place the plates on the tray of the machine.
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 scree

11. After the run is complete, remove the 96 Deep-well Plate from the position. Discard the Magnetic Rod Tip (LA1118B). Dispense the eluted nucleic acid from column 6 and column 12 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 plates as follows:

1. Switch on the UV light for 10 minutes prior to use.
2. Open the door of Insta NX® Mag16 machine.
3. Select **"M583TUCS"** program.
4. Remove the seal from the **Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).**

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

5. Add **50µl of Elution Buffer (ET) [10mM Tris-Cl, pH8.5] (DS0040)** into the **6th and 12th column of the Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).**
6. **Add 20µl of Proteinase K (DS0013) and 200µl pre- processed sample in the 1st and the 7th column of the Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).**

    **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 and the 7th column of the Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).

7. **Add 20µl Magnetic Beads (DS1005A) in the 1st and the 7th column of the Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).**

    **NOTE: 16 samples can be processed in a single Pre-filled Plates for Clinical Multi- purpose Purification (PF16F).**

8. After adding the above solutions place the plates on the tray of the machine.

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 the 96 Deep-well Plate from the position. Discard the Magnetic Rod Tip (LA1118B). Dispense the eluted nucleic acid from column 6 and column 12 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.

**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 is tested against predetermined specifications to ensure consistent product quality.

**Safety Information**

The HiPurA® Pre- filled Clinical Multi- purpose Nucleic Acid Purification Kit 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 of in accordance with current laboratory techniques.

**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 | | |

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