MB531 HiPurA™ Buccal DNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB531		
		20 Preps	50 Preps	250 Preps
ML116	Resuspension Solution (1X PBS)	10 ml	24 ml	120 ml
DS0010	Lysis Solution (C1)	10 ml	24 ml	120 ml
DS0011	Prewash Solution Concentrate (PW)	6 ml	12 ml	60 ml
DS0012	Wash Solution Concentrate (WS)	4 ml	8 ml	40 ml
DS0040	Elution Buffer (ET) [10mM Tris-Cl, pH 8.5]	10 ml	26 ml	120 ml
MB086	Proteinase K	10 mg	24 mg	120 mg
DS0003	RNase A Solution (20 mg/ml)	0.45 ml	1.1 ml	5.5 ml
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	22 nos	55 nos	260 nos
DBCA016	Collection Tubes (Uncapped), Polypropylene (2.0 ml)	20 nos	50 nos	250 nos
PW1139	Collection Tubes, Polypropylene (2.0 ml)	40 nos	100 nos	2 X 250 nos

Introduction

HiPurA Buccal DNA Purification Kit provides a fast and easy method for purification of total DNA for reliable applications in PCR and Southern blotting technique, etc. The DNA purification procedure using the miniprep spin column comprises of three steps viz. adsorption of DNA to the membrane, removal of residual contaminants and elution of pure genomic DNA. HiMedia's HiElute Miniprep Spin Column (Capped) format allows rapid processing of multiple samples. The columns have a high binding capacity and high quality DNA is obtained from various species. The DNA obtained is compatible with downstream applications such as restriction enzyme digestion, PCR and Southern blotting.

HiPurA™ Buccal DNA Purification Kit

This kit simplifies isolation of DNA from the inside of each cheek or gargled samples with spin column procedure. Following lysis, DNA binds to silica gel membrane of the HiElute Miniprep Spin Column (Capped) to yield purified of DNA. Two rapid wash steps remove trace salts and protein contaminants resulting in the elution of high quality DNA in the Elution Buffer (ET) provided with the kit. Typical yield of DNA is 0.5-3.5 µg of DNA.

HiElute Miniprep Spin Column (Capped) [DBCA03]

HiElute Miniprep Spin Column (Capped) is based on the advanced silica binding principle presented in a microspin format. The system efficiently couples the reversible nucleic acid-binding properties of the advanced gel membrane and the speed plus versatility of spin column technology to yield high quantity of DNA. The use of spin column facilitates the binding, washing and elution steps thus enabling multiple samples to be processed simultaneously. This column eliminates the need for alcohol precipitation, expensive resins,

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and harmful organic compounds such as phenol and chloroform, otherwise employed in traditional DNA isolation techniques. DNA binds specifically to the advanced silica-gel membrane while contaminants pass through. PCR inhibitors such as divalent cations and proteins are completely removed in two efficient wash steps, leaving pure nucleic acid to be eluted in the buffer provided with the kit. The purified DNA is upto 20-30 kb in length and can be used for further downstream applications.

Elution

A single elution with 150 µl of Elution Buffer (ET) or Molecular Biology Grade Water will provide sufficient DNA to carry out multiple amplification reaction. Elution with volume less than 150 µl will increase the final DNA concentration, but will reduce the overall DNA yield.

Concentration, yield and purity of DNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the genomic DNA. Use Elution Buffer (ET) to dilute samples and to calibrate the spectrophotometer, measure the absorbance at 260 nm, 280 nm, and 320 nm using a quartz microcuvette. Absorbance readings at 260 nm should fall between 0.1 and 1.0. The 320 nm absorbance is used to correct for background absorbance. An absorbance of 1.0 at 260 nm corresponds to approximately 50µg/ml of DNA. The $A_{260}–A_{320}$ /$A_{280}–A_{320}$ ratio should be 1.6–1.9. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. DNA purified by HiPurA Buccal DNA Purification Kit is free of protein and other contaminants that can inhibit PCR or other enzymatic reactions.

Concentration of DNA sample (µg/ml) = 50 x A_{260} x dilution factor.

Materials needed but not provided

- Ethanol (96-100%)
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Molecular Biology Grade Water (Product code: ML024)
- 55^{o}C water bath or heating block
- Sterile foam Tipped Swab (Product Code: PW1174) for collection of sample from inside of cheek

S0torage

Store the HiPurA Buccal DNA Purification Kit between 15-25°C except certain components as specified on each labels. Under recommended condition kit is stable for 1 year.

General Preparation Instructions

1. Preheat a water bath or heating block at 55°C.
2. **Thoroughly mix reagents.**
   Examine the solutions for any kind of precipitation. If any solution (except RNase A) forms a precipitate, warm at 55-65°C until the precipitate dissolves completely, allow it to cool to room temperature (15-25°C) before use.
3. Ensure that clean & dry tubes and tips are used for the procedure.
4. **Dilute Prewash Solution Concentrate (PW) (DS0011) as follows:**

Number of Preps	Prewash Solution Concentrate (PW)	Ethanol (96-100%)
20	6 ml	9 ml
50	12 ml	18 ml
250	60 ml	90 ml

5. **Dilute Wash Solution Concentrate (WS) (DS0012) as follows:**

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100%)
20	4 ml	12 ml
50	8 ml	24 ml
250	40 ml	120 ml

6. **Reconstitute Proteinase K (MB086)**

The HiPurA Buccal DNA Purification Kit contains Proteinase K. Intensive research has shown that it is the optimal enzyme for use with the Lysis Solution provided in the kit. It is completely free of DNase and RNase activity. Proteinase K is the enzyme of choice for use with an SDS containing Lysis Solution. The specific activity of Proteinase K is 33.5 units/mg dry weight.

Resuspend the Proteinase K (MB086) powder in Molecular Biology Grade Water (ML024) to obtain a 20 mg/ml stock solution.

Number of Preps	Proteinase K	Molecular Biology Grade Water
20	10 mg	0.5 ml
50	24 mg	1.20 ml
250	120 mg	6 ml

The product as supplied is stable at room temperature, upon reconstitution store at -20°C as mentioned in storage instructions.

NOTE: The Proteinase K solution must be added directly to each sample preparation every time. Do not combine the Proteinase K and Lysis solutions for storage.

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 260nm 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).

Centrifugation

All centrifugation steps are carried out in conventional laboratory centrifuge e.g. Beckman CS-6KR, Heraeus Varifuge 3.0R, or Sigma 6k10 with fixed angle rotor. The tubes provided with the kit are compatible with almost all laboratory centrifuges and rotors. All centrifugation steps are performed at room temperature and are given in g, the correct rpm can be calculated using the formula:

$$RPM = \sqrt{RCF/1.118 \times 10^{-5} r}$$

where *RCF* = required gravitational acceleration (relative centrifugal force in units of g); *r* = radius of the rotor in cm; and *RPM* = the number of revolutions per minute required to achieve the necessary *g*-force.

Procedure

NOTE: We recommend using Sterile foam Tipped Swab (Product Code: PW1174) 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 Resuspension Solution (1X PBS) (ML116) to the tube.

2. Centrifuge the tube at ≈14,000 rpm for 5 minutes. Discard the pellet and transfer the supernatant to a new collection tube.

3. Add 20 µl of the Proteinase K solution (20 mg/ml) **(Refer to General Preparation Instructions)** into the tube containing the supernatant. Vortex for 10-15 seconds to ensure thorough mixing of enzyme.
   NOTE: If residual RNA is not a concern, continue with lysis step.

   Optional RNase A treatment
   If RNA-free genomic DNA is required, add 20 µl of RNase A solution (20 mg/ml) (DS0003). Vortex for 10-15 seconds and incubate for 2 minutes at room temperature (15-25°C); continue with lysis step.

4. **Lysis reaction**
   Add 400 µl Lysis Solution (C1) (DS0010) to the tube, vortex thoroughly for a few seconds to obtain a homogenous mixture. Incubate at 55°C for 10 minutes.
   NOTE: If clumps are visible, the sample can be pipetted gently to obtain a homogenous mixture.

5. **Prepare for Binding**
   Add 400 µl of ethanol (96-100%) to the lysate obtained from the above step. Mix thoroughly by vortexing for 5-10 seconds.

   NOTE: A homogenous solution is essential.

6. **Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Transfer the lysate obtained from Step 4 into HiElute Miniprep Spin Column (Capped) provided. Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute. Discard the flow-through liquid.

   NOTE: Use a wide bore pipette tip to reduce shearing of the DNA when transferring contents into the column.

7. Repeat the above step with any remaining lysate. Discard the flow-through liquid and place the column in a same 2.0 ml collection tube.

8. **Prewash (PW) (DS0011)**
   (Prepare Prewash Solution as indicated in General Preparation Instructions)
   Add 500 µl of diluted Prewash Solution to the column and centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute. Discard the flow-through liquid and re-use the same collection tube with the column.

9. **Wash (WS) (DS0012)**
   (Prepare Wash Solution as indicated in General Preparation Instructions)
   Add 500 µl of diluted Wash Solution to the column and centrifuge at 12,000-16,000 x g (≈13,000-16,000 rpm) for 3 minutes to dry the column. Discard the flow-through. Centrifuge the column for another minute at the same speed if residual ethanol is observed. Discard the flow-through liquid and place the column in a new uncapped 2.0 ml

collection tube.

NOTE: The column must be free of ethanol before eluting the DNA. The tube can be emptied and re-used for this additional centrifugation step.

10. **DNA Elution**
    Pipette 150 µl of the Elution Buffer (ET) (DS0040) directly onto the column without spilling to the sides. Incubate at 1 minute at room temperature. Centrifuge at ≥6500 x g (≈10,000rpm) for 1 minute to elute the DNA.

    Optional: A second elution can be performed by repeating the above step.

    NOTE: To increase the elution efficiency, incubate for 5 minutes at room temperature (15-25°C) after adding the Elution Buffer (ET), then centrifuge. Elution with volume less than 150 µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield. Storing DNA in water can cause acid hydrolysis.

Storage of the eluate with purified DNA: The eluate contains pure genomic DNA. For short-term storage (24-48 hrs) of the DNA, 2-8°C is recommended. For long-term storage, -20°C or lower temperature (-80°C) is recommended. Avoid repeated freezing and thawing of the sample which may cause denaturing of DNA. The Elution Buffer will help to stabilize the DNA at these temperatures.

Quality Control

Each lot of HiMedia's HiPurA Buccal DNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

References

1. Sambrook, J.,*et al*. Molecular Cloning: A laboratory Manual, 2nd ed. (Cold Spring Harbour Laboratory Press, Plainview, NY, 1989).

Troubleshooting guide:

Sr.No.	Problem	Possible Cause	Solution
1.	Spin column is clogged	Sample is too large	Use smaller quantity of sample. Clogging also can be alleviated by increasing the g- force or spinning for longer time until the lysate passes through the binding column. The yield of genomic DNA reduces.
		Inefficient disruption of buccal cells	The Proteinase K digestion at 55°C can be extended. Invert the sample tube after Proteinase K digestion for a homogenous mixture. The Proteinase K solution must be added directly to each sample preparation every time. Do not combine the Proteinase K and Lysis Solutions for storage.

		Lysate/ethanol mixture is not homogenous	Vortex the tubes for atleast 5-10 seconds in order to obtain a homogenous solution before applying it to the column. If minimally sheared DNA is required for downstream applications like PCR, mix with gentle pipetting or inversion until
			homogenous instead of vortexing.
		DNA elution is improper	Ensure that the DNA elution is in 150 µl of Elution Buffer. To improve the DNA yield, incubate for 5 minutes at room temperature after Elution Buffer is added to the column.
		Ethanol was omitted during binding	Ensure that ethanol is added in step 4 before adding the sample to the spin column.
		Eluate contains residual ethanol from the wash	Remove ethanol from the second wash completely before eluting the DNA. Spin for an additional 2 minutes to dry the membrane completely. In order to avoid the interference of ethanol, always use a fresh tube for elution.
		Use of water instead of Elution Buffer for elution of DNA	Elution Buffer is recommended for optimal yields and storage of the genomic DNA. If water is used instead of the Elution Buffer the pH should be at least 7.0, to avoid acidic conditions which may cause acid hydrolysis of DNA when stored for long periods of time. **NOTE:** Only DNase/RNase and Protease free water should be used for eluting DNA
2.	Purity of the DNA is lower than expected; (A_{260}/A_{280} ratio is low)	Eluate was diluted in water for absorbance measurement	Use either the Elution Buffer provided, or 10 mM Tris-HCl, pH 8.0-8.5.
		Background reading is high due to silica fines	Spin the DNA sample at maximum speed for 1 minute, the supernatant can be used to repeat the absorbance readings.
3.	Purity of the DNA is higher than expected;(A_{260}/A_{280} ratio is too high)	RNA contamination	RNase A treatment can be included in future isolations or the final product can be treated with RNase A Solution and repurified.

4.	Shearing of genomic DNA	Improper handling of genomic DNA	All pipetting steps should be executed as gently as possible. Wide orifice pipette tips are recommended to eliminate shearing of the DNA to a large extent. If the isolated DNA is to be used for PCR, instead of vortexing mix by gentle pipetting
			or invert until homogenous. This reduces shearing of DNA considerably.
		Sample is old, degraded, or has undergone repeated freeze/ thaw cycles	Fresh cells should be used immediately. Old material may yield degraded DNA in the eluate. Cells can be frozen in liquid nitrogen and stored at -70°C until needed.
5.	Downstream applications are inhibited	Traces of ethanol present in the final genomic DNA preparation	After the washing steps the eluate should not come in contact with the column. Spin the column for 1 minute at maximum speed (12,000-16,000 x g) if necessary, after emptying the collection tube.
		Salt is carried over in the final genomic DNA preparation	The spin column should be transferred to a new 2.0 ml collection tube before adding the wash solutions.

Safety Information

The HiPurA Buccal DNA 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 Material Safety Data Sheet (MSDS) for information regarding hazards and safe handling practices.

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.

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