MB562 HiPurA® Food DNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB562		
		20 Preps	50 Preps	250 Preps
DS0010	Lysis Solution (C1)	20 ml	50 ml	250 ml
DS0094	Binding solution (FB)	20 ml	50 ml	250 ml
DS0011	Prewash Solution Concentrate (PW)	6 ml	15 ml	75 ml
DS0012	Wash Solution Concentrate (WS)	4 ml	10 ml	50 ml
DS0040	Elution Buffer (ET) [10 mM Tris-Cl, pH 8.5]	6 ml	15 ml	75 ml
DS2280	Proteinase K	10 mg	25 mg	125mg
DS0003	RNase A Solution (20 mg/ml)	0.5 ml	1.25 ml	6.25 ml
DBCA03	HiElute Miniprep Spin Column (Capped) [in DBCA016 Collection Tube]	20 nos	50 nos	250 nos
DBCA016	Collection Tubes (Uncapped), Polypropylene (2.0 ml)	20 nos	50 nos	250 nos
DBCA017	Collection Tubes, Polypropylene (2.0 ml)	40 nos	100 nos	2 X 250 nos

Intended Use

Recommended for isolation of DNA from Food samples.

Introduction

HiPurA® Food DNA Purification Kit provides a fast and easy method for purification of total DNA from various sources of food samples for downstream applications such as PCR, RT-PCR, 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 genomic DNA is obtained from various species. The DNA obtained is compatible with downstream applications such as restriction enzyme digestion, PCR and Southern blotting.

HiPurA® Food DNA Purification Kit

This kit simplifies isolation of DNA from variety of raw and processed food matrices. Genomic DNA purification from food involves cell lysis, which is achieved by incubation of food in a solution containing chaotropic ions in the presence of Proteinase K at 65°C. Following lysis, DNA binds to the silica gel membrane of the HiElute Miniprep Spin Column (Capped) to yield purified DNA. After the initial binding of DNA, impurities like proteins, polysaccharides, low molecular weight metabolites and salts are removed by short washing steps. High quality DNA is finally eluted in the Elution Buffer provided with the kit.

HiElute Miniprep Spin Column (Capped) [DBCA03]

HiElute Miniprep Spin Column (Capped) is based on the advanced silica binding principle presented in a microspin format.

ISO 9001-2015 CERTIFIED
WHO GMP CERTIFIED
ISO 13485-2016 CERTIFIED

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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 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 can be used for further downstream applications.

Elution

The yield of genomic DNA depends on the sample type and the number of cells in the sample. An elution with 50 µl of Elution Buffer (ET) will provide sufficient DNA to carry out multiple amplification reactions. Elution with volume less than 50 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 50 µg/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® Food 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

- 65°C water bath or heating block
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Ethanol (96-100%)
- Molecular Biology Grade Water (Product code: ML024)
- Mortar and pestle
- Liquid nitrogen

Storage

Store the HiPurA® Food DNA Purification Kit should be stored between 15-25°C except certain components as specified on each labels. Under recommended condition kit is stable for 18 months.

General Preparation Instructions

1. Preheat a water bath or heating block to 65°C.

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. The reagent should be at 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	15 ml	22.5 ml
250	75 ml	112.5 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	10 ml	30 ml
250	50 ml	150 ml

6. **Reconstitute Proteinase K (DS2280)**

   The HiPurA® Food 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 (DS2280) 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	25 mg	1.25 ml
250	125 mg	6.25 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 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).

Centrifugation

All centrifugation steps are carried out in conventional laboratory 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.

Specimen Collection and Handling

For Food

Collect food sample in a sterile container and freeze the sample at -20°C for short term storage or -80°C for long term storage.

Types of Specimen

Samples: Food

Procedure

1. **Sample Preparation and cell lysis**

a. Solid Sample: Grind the sample to fine powder using commercial homogenizer or liquid nitrogen in a mortar and pestle.

NOTE: Lysis is more efficient when the sample is well homogenized. Homogenization is not needed; however, complete suspension is required for efficient lysis).

Transfer 25-200mg of ground sample into a clean 2.0ml capped microcentrifuge tube. Add 700 µl Lysis Solution C1 (DS0010) and 20 µl of the reconstituted Proteinase K solution (20 mg/ml) **(Refer to General Preparation Instructions)**. Vortex for 10-15 seconds to ensure thorough mixing. Incubate at 65°C for 30 minutes with occasional mixing during incubation to ensure thorough lysis of sample.

b. Liquid Sample: Transfer 400 µl of sample directly into clean 2.0ml capped microcentrifuge tube. Add 300 µl of Lysis Solution C1 (DS0010) and 20 µl of the reconstituted Proteinase K solution (20 mg/ml) **(Refer to General Preparation Instructions).** Vortex for 10-15 seconds to ensure thorough mixing. Incubate at 65°C for 30 minutes with occasional mixing during incubation to ensure thorough lysis of sample.

Optional RNase A treatment

If RNA-free 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 step 2.

2. Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute to pellet down the cell debris and contaminants. Transfer 450µl of the cleared supernatant to a clean micro centrifuge tube (not provided). Add 1.5 volumes of Binding solution (FB) (DS0094) and mix thoroughly by pulse vortexing until a homogenous solution is obtained. Incubate at 65°C for 10 minutes.

NOTE: While transferring the supernatant, avoid touching the pellet and the layer of contaminants on top of the solution if any. If the supernatant transferred is less than 450 µl adjust the volume of FB Solution to be added proportionately.

3. **Prepare for Binding**

Add 350 µl of ethanol (96-100%) to the lysate obtained from the above step for preparation of lysate for binding to the spin column. Mix thoroughly by gentle pipetting or pulse vortexing.

NOTE: A homogenous solution is essential.

4. **Load lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
   Transfer 750 µl of the lysate obtained from the above step into the spin column provided. Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute. Discard the flow-through liquid and place the column in a same 2.0 ml collection tube.

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

5. Repeat the above step with the remaining sample. Discard the flow-through liquid.

6. **Prewash**
   (Prepare Prewash Solution Concentrate (PW) (DS0011) 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.

7. **Wash**
   (Prepare Wash Solution Concentrate (WS) (DS0012) 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 liquid and spin the empty column for another minute at the same speed if any residual ethanol is observed. Discard the collection tube containing the flow-through liquid and place the column in a new 2.0 ml uncapped 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.

8. **DNA Elution**
   Pipette 50 µl of the Elution Buffer (ET) (DS0040) directly onto the column without spilling to the sides. Incubate for 1 minute at room temperature (15-25°C). Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute to elute the DNA.

   NOTE: To increase the elution efficiency, incubate for 5 minutes at room temperature after adding the Elution Buffer (ET) and then centrifuge. Elution with volumes less than 100 µl increases the final DNA concentration in the eluate significantly, but slightly reduces the overall DNA yield.

9. Transfer the eluate to a fresh capped 2ml collection tube for longer DNA storage

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.

Warning and Precautions

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

Limitations

1. The yield of DNA depends upon the type and the volume of starting material used.

Performance and Evaluation

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

Quality Control

Type of Sample	DNA Purity
Milk	1.6-1.9

Troubleshooting guide

Sr. No.	Problem	Possible Cause	Solution
1.	Spin column is clogged	Lysate/ethanol mixture is not homogenous	Vortex the tubes for atleast 5-10 seconds in order to obtain 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 100 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 skipped during binding	Ensure that ethanol is added in step 3 before adding the sample to the HiElute Miniprep Spin Column (Capped) in step 4.
		Eluate contains residual ethanol from the wash	Remove ethanol from the second wash completely before eluting the DNA. Spin for 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 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.
		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.	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 re-purified.
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 with gentle pipetting or invert until homogenous. This reduces shearing of DNA considerably.
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 2 minutes at maximum speed 12,000-16,000 x g (≈13,000-16,000 rpm) if necessary, after emptying the collection tube.
		Salt is carried over in the final eluate containing DNA	The column should be transferred to a new 2.0 ml collection tube before adding the wash solution in steps 10-11.

Safety Information

The HiPurA® Food DNA Purification Kit is for laboratory use only, not for drug, household or other uses. The Lysis Solution (C1) contains chaotropic salts, which are irritants. 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.

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
	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)		Catalogue number

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