MB527 HiPurA® Streptomyces DNA Purification Kit

Kit Contents

Product Code	Reagents provided	MB527		
		20 Preps	50 Preps	250 Preps
DS0015	Lysis Solution (AL)	8 ml	20 ml	100 ml
DS0010	Lysis Solution (C1)	6 ml	15 ml	75 ml
DS0031	Prewash Solution (PWB)	12 ml	30 ml	150 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
MB086	Proteinase K	10 mg	25 mg	125 mg
DS0003	RNase A Solution (20 mg/ml)	0.6 ml	1.5 ml	7.5 ml
DBCA03	HiElute Miniprep Spin Column(Capped) [in DBCA016 Collection Tube]	20 nos	50 nos	250 nos
DBCA016	Collection Tube(Uncapped) , Polypropylene (2.0 ml)	20 nos	50 nos	250 nos
DBCA05	HiBead™ Tube	20 nos	50 nos	250 nos
PW1139	Collection Tube, Polypropylene (2.0 ml)	60 nos	150 nos	3 X 250 nos

Introduction

HiPurA® Streptomyces DNA Purification Kit provides a fast and easy method for purification of total DNA for reliable applications in PCR and Southern blotting technique. The DNA purification procedure using the miniprep spin columns 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® Streptomyces DNA Purification Kit

This kit simplifies isolation of DNA from *Streptomyces* by the spin-column procedure. *Streptomyces* is grown in the medium till it reaches log phase and cell mass harvested by centrifugation. After harvesting, the cell wall is degraded by Proteinase K. Following lysis is the binding of DNA to the silica-gel membrane of the HiElute Miniprep Spin Column (Capped) to yield approximately 15-20 µg of purified DNA. Two rapid wash steps removes trace salt and protein contaminants resulting in the elution of high quality DNA in the Elution Buffer provided with the kit.

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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 silica 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 is upto 20-30 kb in length and 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. A single elution with 200 µl of Elution Buffer (ET) will provide sufficient DNA to carry out multiple amplification reaction. Elution with volume less than 200 µl will increase the final DNA concentration, but will reduce the overall DNA yield. The eluted DNA ranges in size upto 20-30 kb, and is suitable for direct use in PCR, restriction digestion, and Southern blotting applications.

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® Streptomyces 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:

- 55°C water bath or heating block
- 95°C water bath
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- Ethanol (96 – 100%)
- Potato Dextrose Broth for cultivation of *Streptomyces* (Product code: M403)
- Molecular Biology Grade Water (Product code: ML024)

Storage

Store the HiPurA® Streptomyces 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 to 55°C and 95°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 and allow cooling to room temperature (15-25°C) before use.

3. Ensure that clean & dry tubes and tips are used for the procedure.

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

Number of Preps	Wash Solution Concentrate (WS)	Ethanol (96-100 %)
20	4 ml	16 ml
50	10 ml	40 ml
250	50 ml	200 ml

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

   The HiPurA® Streptomyces 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 powder in Molecular **B**iology 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 (15-25°C), 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 Solution 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 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

1. **Harvesting of cells**
   Pellet freshly sub cultured *Streptomyces* in 2ml collection tube (not provided) by centrifuging for 2 minutes at 12,000-16,000 x g (≈14,000 rpm) to obtain 10-15 mg (wet weight). Remove the culture medium and discard.

2. **Resuspend cells**
   Resuspend the fungi thoroughly in 300 µl of Lysis Solution (AL) (DS0015). If residual RNA is not a concern, continue with step 3.

   Optional RNase A treatment
   If RNA- free genomic DNA is required add 20 µl of RNase A Solution (DS0003), mix and incubate for 2 minutes at room temperature (15-25°C), then continue with step 3.

3. **Prepare for cell lysis**
   Add 20 µl of the Proteinase K Solution (20 mg/ml) (DS0013) to the sample. Mix and transfer the resuspended cells to HiBead Tube (DBCA05). Incubate for 30 minutes at 55°C.

4. Secure HiBead tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 5-7 minutes.

5. Incubate for 10 minutes at 95°C followed by pulse vortexing (once or twice).

6. Make sure the 2.0 ml HiBead Tubes rotate freely in the centrifuge without rubbing. Centrifuge the tubes at 10,000 x g (13,000 rpm) for 1 minute at room temperature.

7. Transfer the supernatant to a clean capped 2.0 ml collection tube.

8. **Lyse cells**
   Add 200 µl of Lysis Solution (C1) (DS0010), vortex thoroughly (for about 15 seconds) and incubate at 55°C for 10 minutes.

   NOTE: A homogeneous mixture is essential for efficient lysis.

9. **Prepare for binding**
   Add 200 µl of ethanol (96-100%) to the lysate and mix thoroughly by pipetting or vortexing for 15 seconds.

   NOTE: A homogenous mixture is essential. A white precipitate may form on addition of ethanol. It is essential to apply all of the precipitate to the HiElute Miniprep Spin Column (Capped). This precipitate does not interfere with the DNA isolation procedure or with any subsequent applications. Do not use alcohols other than ethanol because this may result in reduced yields.

10. **Load lysate in HiElute Miniprep Spin column (Capped) [DBCA03]**
    Transfer the lysate obtained from the above step into HiElute Miniprep Spin column (Capped) provided. Centrifuge at ≥6,500 x g (≈10,000 rpm) for 1 minute. Discard the flow-through liquid and place the spin 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. It is essential to apply all of the precipitate to the HiElute

Miniprep Spin Column. If the solution has not completely passed through the membrane, centrifuge again at a higher speed until all the solution has passed through. Centrifugation at full speed will not affect the yield or purity of the DNA.

11. **Prewash**
    Add 500 µl of Prewash Solution (PWB) (DS0031) 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.

12. **Wash**
    (Prepare Wash Solution as indicated in General Preparation Instructions)
    Add 500 µl of Wash Solution Diluted (WS) (DS0012) to the column and centrifuge for 3 minutes at maximum speed 12,000-16,000 x g (≈13,000-16,000 rpm) to dry the column. The column must be free of ethanol before eluting the DNA. Discard the flow-through and reuse the same collection tube. Centrifuge the column for the additional 1 minute at maximum speed if residual ethanol is seen.

13. **DNA Elution**
    Discard the flow-through and transfer the column into to new uncapped collection tube. Pipette 200 µl of the Elution Buffer (ET) (DS0040) directly into the column without spilling to the sides. Incubate for 1 minute at room temperature. 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), then centrifuge. Elution with volumes less than 200 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.

14. Transfer the eluate to a fresh capped 2ml collection tube for longer 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.

Precautions

Read the procedure carefully before starting the experiment.

Performance and Evaluation

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

Quality Control

Type of Sample	DNA Yield	DNA Purity
Streptomyces culture	15- 20 µg	1.6-1.9

References

1. Sambrook, J., *et al*. Molecular Cloning: A laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Plainview, NY, 1989).
2. Birren, B. and Lai, E. Pulsed Field Gel Electrophoresis: A practical guide (Academic Press, San Diego, CA, 1993).

Troubleshooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1.	HiElute Miniprep Spin column (Capped) is clogged	Sample volume is large	Use smaller quantity of sample to salvage the current preparation, clogging can be alleviated by increasing the g-force or spinning for longer time until the lysate passes through the spin column.
2.	Lysate appears to be gelatinous prior to loading onto the column	Sample volume is large	Use only upto 15 mg cell mass. The incubation time and or the amount of Proteinase K solution can be increased.
3.	Poor / Lower yield of genomic DNA	Sample is old	It is necessary to use cells before they reach their maximum density or they become confluent.
		Incomplete lysis of cells.	It is necessary to use cells before they reach their maximum density or they become confluent.
		Incomplete lysis of cells.	The incubation time and or the amount of Proteinase K solution can be increased.
		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.
		DNA Elution is incomplete	DNA yield can be improved by incubating the Elution Buffer for 5 minutes at room temperature (15-25°C) after it is added to the column.
		Eluate contains residual ethanol from the wash solution	Ethanol from the final wash should be eliminated completely before eluting DNA. Spin the tubes for longer time to dry the column completely.
		Wash Solution Concentrate was not diluted before use	Check that the Wash Solution Concentrate is properly diluted with ethanol as per instructions.
		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.
4.	Purity of the DNA is	Background	The DNA sample can be

	lower than expected; A_{260}/A_{280} ratio is low	reading is high due to silica fines	centrifuged at maximum speed for 1 minute, the supernatant can be used to repeat the absorbance readings.
		Sample diluted in water	Use Elution Buffer provided, as the eluant.
5.	Purity of the DNA is lower than expected; A_{260}/A_{280} ratio is too high	RNA contamination	RNase A treatment should be included in future isolations or the final product can be treated with RNase A and repurified.
6.	DNA is sheared	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, mix with gentle pipetting or invert until homogenous, instead of vortexing as it reduces shearing of DNA considerably.
		Cells are old	Cells grown for a longer time period may lyse prematurely when subjected to cell wall lysing enzyme, which may result in the release of endogenous nucleases and subsequent DNA degradation.
7.	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 (12,000-16,000xg) if necessary, after emptying the collection tube.
		Salt is carried over in the final genomic DNA preparation	The HiElute Miniprep Spin Column (Capped) should be transferred to a new 2.0 ml collection tube before adding the wash solution.

Safety Information

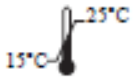
The HiPurA® Streptomyces DNA Purification Kit is for laboratory use only, not for drug, household or other uses. The Lysis Solution (C1) contains chaotropic salts, which are irritants. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfecting agents containing 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.



Storage temperature

Do not use if package is damaged

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