

MB503

HiPurA® Silica Kit for DNA Isolation

Kit Contents

| Product Code | Reagents provided | MB503 |
|--------------|----------------------------------|-----------|
| | | 400 Preps |
| DS0006 | Glass Powder Suspension (GPS) | 8 ml |
| DS0007 | Chaotropic Salt Solution (CSS) | 2X 240 ml |
| DS0008 | Concentrated Wash Solution (SWS) | 40 ml |

Intended Use

Recommended for isolation of DNA from Plasmid, tissue, agarose and from PCR reactions

Introduction

The HiPurA® Silica Kit for DNA Isolation provides a convenient method for extracting DNA from low melting agarose gels, for recovering DNA from Polymerase Chain Reactions or from tissues treated with various chemicals, isolating DNA from mini-preps, as well as for concentration of DNA without ethanol precipitation.

Principle

This method involves the use of chaotropic salts that can solubilize agarose at 55°C. In the presence of high concentrations of chaotropic salts, DNA binds to glass powder particles and elutes when the salt concentration is reduced. As the salt concentration reduces, rehydration of the DNA breaks the bonding between the glass powder matrix and the DNA. The glass powder with adsorbed DNA when washed with the wash buffer containing ethanol helps in the removal of salt and impurities from the original sample. The clean DNA can be eluted in water or TE buffer at 55°C. This isolation method of DNA is faster and easier to perform than the other organic solvent based extraction methods.

Materials needed but not provided

- Molecular Biology Grade Water (Product Code: ML024)
- For protocol B
 - 1) TGE Buffer (25 mM Tris, pH 8.0, 50 mM Glucose, 10 mM EDTA)
(Product code: ML013-1M Tris- Cl pH 8.0)
 - 2) 0.2N NaOH + 1% SDS Solution (Product code: ML007- 20% SDS stock solution)
 - 3) Potassium acetate Solution (3M with respect to potassium, 5M with respect to acetate) (Product code: ML006)
- For protocols C and D
 - 1) 6 M Guanidine hydrochloride (Product code: ML001)
 - 2) Xylene
- For protocols A, B, C and D
 - 1) TE buffer (pH 8.0) [10mM Tris-HCl, 1mM EDTA, pH 8.0]
 - 2) (96-100%) Ethanol

Storage Conditions

HiPurA® Silica Kit for DNA Isolation should be stored at 2-8°C. The diluted Wash Solution should be stored at -20°C.

Concentration, yield and purity of DNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and 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® Silica Kit for DNA Isolation 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.

Preparation and Storage of Reagents

1. Glass Powder Suspension (GPS): Store tightly sealed with parafilm at 2-8°C. Mix well before using. If the Glass Powder suspension loses liquid and dries out, add sterile Molecular Biology Grade Water (ML024) such that the glass powder suspension accounts for approximately two-thirds of the total volume.
2. Chaotropic Salt Solution (CSS): Store at 2-8°C protected from light.
3. Concentrated Wash Solution (SWS): Dilute the Concentrated Wash Solution by adding 360 ml of sterile Molecular Biology Grade Water (ML024) to 40 ml Concentrated Wash Solution (SWS) (DS0008), and add 400 ml of Ethanol (96-100%). After dilution, store at -20°C in a glass bottle.

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. 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 Plasmid

Collect overnight culture from sterile flask with the help of micropipette. Store the remaining culture at 2-8°C for short term use.

For tissues

Collect human/animal cells, tissues, blood sample in a sterile container and freeze the sample at -20°C for short term storage or -80°C for long term storage. Ensure that the tissue is at room temperature before beginning the protocol.

Types of Specimen

Samples: Plasmid cultures, tissues, DNA in agarose gel and PCR products

PROTOCOLS FOR DNA ISOLATION

A. Isolation of DNA from Agarose

NOTE: WE RECOMMEND USING LOW MELTING AGAROSE AND TAE BUFFER FOR OPTIMUM RESULTS.

1. Run the agarose gel for the DNA to be purified. Excise the DNA bands from the ethidium bromide stained gel with a clean razor blade or scalpel blade using 312 nm UV light. If necessary, store it in a plastic microcentrifuge tube at 4°C.
2. Quantitate the concentration of the DNA using a spectrophotometer or estimate the concentration by comparing its intensity with that of a DNA ladder of known concentration.
3. Determine the approximate weight of the gel slice and accordingly add 3 volumes of Chaotropic Salt Solution (CSS) (DS0007) per gel slice volume. Incubate at 55°C for 5-10 minutes; mix the contents of the tube every 2-3 minutes so that the agarose is completely dissolved.
4. Vortex the Glass Powder Suspension (GPS) (DS0006) thoroughly. Add 10 µl of well-mixed glass powder suspension to the Agarose: DNA: Chaotropic Salt Solution and incubate at room temperature (15-25°C) for 10 minutes with occasional mixing every 2-3 minutes for the glass powder to be completely resuspended in the solution. Centrifuge at $\geq 6,500 \times g$ ($\approx 10,000$ rpm) for 10 seconds.

NOTE: The supernatant solution can be saved to be used later in case the DNA elution is not proper.

5. Rinse the glass powder pellet with 500 µl of diluted Wash Solution (SWS) (DS0008) (**Refer to Preparation and Storage of Reagents**) and resuspend the glass powder pellet completely by gentle pipetting or pulse vortexing (do not vortex high molecular weight DNA). Centrifuge at $\approx 10,000$ rpm for 1 minute and discard the supernatant. Repeat the washing step two more times.
6. After the last wash remove the supernatant completely using a micropipette tip. If needed, again spin the tube briefly and remove as much of the Wash Solution as possible. Air dry the pellet for 5 minutes.

NOTE: Avoid overdrying, as the DNA will be difficult to resuspend from the pellet.

7. Resuspend the glass powder pellet in 15-50 µl of TE buffer of pH 8.0. Incubate at 55°C for 8-10 minutes with intermittent mixing.
8. Mix and centrifuge at $\approx 10,000$ rpm for 1 minute and then carefully transfer the supernatant containing the DNA to a new tube and again centrifuge at $\approx 10,000$ rpm for 1 minute to remove the traces of Glass Powder Suspension (GPS) completely (this also allows easy evacuation of the supernatant). Carefully transfer the DNA to a new tube.

B. Isolation of Plasmid DNA from Overnight Culture

1. Inoculate 5 ml of the transformed culture, incubate overnight.
2. Add 1.5 ml of the culture into a microcentrifuge tube and pellet the cells at $\approx 12,000$ rpm for 3 minutes.

3. Discard the supernatant and resuspend pellet in 100 μ l of chilled TGE Buffer (25 mM Tris, pH 8.0, 50 mM Glucose, 10 mM EDTA). Add 200 μ l of 0.2N NaOH + 1% SDS solution. Mix gently by inverting (do not vortex). Keep on ice for 3-5 minutes.
4. Add 150 μ l of cold potassium acetate solution (3M with respect to potassium, 5M with respect to acetate) (ML006).
5. Mix gently by inverting (do not vortex). Keep on ice for 3-5 minutes.
6. Centrifuge at maximum speed (\approx 14,000 rpm) for 5 minutes, transfer the supernatant to a clean (2.0 ml) centrifuge tube.
7. Add 3 volumes of Chaotropic Salt Solution (CSS) (DS0007) and mix well.
8. Add 10 μ l of Glass Powder Suspension (GPS) (DS0006), mix well and incubate at room temperature (15-25°C) for 5 minutes. Centrifuge at maximum speed (\approx 14,000 rpm) for 1 minute, discard the supernatant solution.
9. Resuspend the glass powder pellet with 500 μ l of diluted Wash Solution (SWS) (DS0008) (**Refer to Preparation and Storage of Reagents**) by pipetting or by gently flicking the tube. Centrifuge at \approx 10,000 rpm for 1 minute and discard the supernatant. Repeat the washing step two more times.
10. After the last wash, remove the supernatant completely using a micropipette tip. If needed, again spin the tube briefly and remove as much of the Wash Solution as possible. Air dry the pellet for 5 minutes.

NOTE: However, avoid overdrying, as the DNA will be difficult to resuspend from the pellet.

11. Resuspend the glass powder pellet in 15-50 μ l of sterile distilled water or TE Buffer of pH 8.0. Incubate for 3-5 minutes at 55°C with occasional mixing.
12. Centrifuge at maximum speed (\approx 14,000 rpm) for 1 minute. Transfer the supernatant containing the DNA to a new tube. An additional amount of 10-15% of recovered DNA can be obtained from the pellet by repeated elution.

C. Extraction of DNA from Tissues

1. DNA can be recovered from tissues for PCR, which has been treated with chemicals such as paraffin, tissue glue or cover slip glue. For recovery of DNA, the tissue can be treated with toluene, xylene or other organic solvent. (xylene solubilizes majority of chemicals used in glues or paraffin).
2. Resuspend the tissue in a volume of xylene which is 10-fold excess. Incubate at room temperature (15-25°C) for 10 minutes with occasional vortexing. Centrifuge at \approx 10,000 rpm for 1 minute to pellet the tissue. Discard the supernatant.
3. Resuspend the tissue in same volume of xylene as added in step 2, mix by vortexing and centrifuge at \approx 10,000 rpm for 1 minute. Discard the supernatant.
4. Resuspend the tissue in 96-100% ethanol and centrifuge at \approx 10,000 rpm for 1 minute at room temperature (15-25°C). Discard the supernatant. Repeat the washing step once more.

5. After the last wash remove the supernatant completely using a micropipette tip. If needed, again spin the tube briefly and remove as much of the ethanol as possible. Air dry the pellet for 5 minutes.

NOTE: However, avoid overdrying, as the DNA will be difficult to resuspend from the pellet.

6. Add 100 μl of 6M Guanidine hydrochloride, mix well and incubate the tubes at room temperature (15-25°C) for 10-15 minutes to solubilize the proteins and DNA. (If the tissue contains large amounts of keratin, incubate the tubes at 60°C for 10-15 minutes).
7. Add 10 μl of Glass Powder Suspension (GPS) (DS0007), and incubate for 5 minutes at 57°C with occasional mixing every 2-3 minutes to keep glass powder resuspended in solution.
8. Centrifuge the glass powder-DNA complex at $\approx 10,000$ rpm for 1 minute to form a pellet. Discard the supernatant.
9. Add 500 μl of chilled diluted Wash Solution (SWS) (DS0008) (**Refer to Preparation and Storage Instructions**) to the pellet. Resuspend the pellet in the Wash Solution (SWS) by vortexing the pellet. Centrifuge at $\approx 10,000$ rpm for 1 minute, discard the supernatant.
10. Repeat the washing step two more times. During each wash, the pellet should be resuspended completely. After the supernatant from the last wash has been removed, centrifuge again at $\approx 10,000$ rpm for 1 minute to remove the remaining liquid. (Glass pellet should be free from ethanol as it may inhibit the enzyme reactions. It can be air dried or vacuum dried for a few minutes; overdrying may lead to poor recovery).
11. Resuspend the glass powder-DNA complex in water or TE buffer by vortexing in the desired amount of water or TE buffer to be used directly for a PCR reaction. This mixture can be further used for PCR.

NOTE: The same material can be used for several PCR reactions whereby the mixture can be divided into several tubes or the DNA can be eluted with water or TE buffer. Use at least twice the volume of the original glass powder mixture used. Incubate the tube at 57°C for 5 to 10 minutes. Mix and centrifuge for 30 seconds and carefully remove the supernatant containing the DNA, transfer to a fresh tube.

D. Extraction of DNA from Polymerase Chain Reactions

NOTE: For the first 2 μg of DNA add upto 6 μl of the Glass Powder Suspension (GPS) (DS0007) and for every 0.5 μg thereafter, add an additional 1 μl of the Glass Powder Suspension (GPS). Mix well and incubate at room temperature (15-25°C) for 10 minutes and mix for every 2-3 minutes for the glass powder to be completely resuspended in the solution.

1. Centrifuge at $\approx 10,000$ rpm for 1 minute. The supernatant solution can be saved to be used later if the DNA elution is not proper.
2. Rinse the glass powder pellet with 50 volumes of diluted Wash Solution (SWS) (DS0008) (**Refer to Preparation and Storage Instructions**) as compared to the original volume of Glass Powder Suspension (GPS). Resuspend the glass pellet by gentle pipetting or vortexing (do not vortex high molecular weight DNA). Centrifuge at $\approx 10,000$ rpm for 1 minute and discard the supernatant. Repeat the washing procedure two more times.

3. After the last wash, remove the supernatant completely using a micropipette tip. If needed, again spin the tube briefly and remove as much of the Wash Solution as possible. Air dry the pellet for 5 minutes.
4. Resuspend the glass pellet in 1-2 volumes of TE buffer of pH 8.0 as compared to the original volume of the glass powder suspension. Incubate at 55°C for 8-10 minutes with intermittent mixing.
5. Mix and centrifuge at ≈10,000 rpm for 1 minute and then carefully transfer the supernatant containing the DNA to a PCR tube and again centrifuge at ≈10,000 rpm for 1 minute to remove the traces of Glass Powder Suspension completely (this also allows easy evacuation of the supernatant). Carefully transfer the DNA to a new tube.

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® Silica Kit for DNA Isolation is tested against predetermined specifications to ensure consistent product quality.

Quality Control

| Type of Sample | DNA Recovery |
|--------------------|--------------|
| 400mg gel with DNA | 70-80% |

References

1. Wilson, V.G., Bio Techniques6, 733, 1988.
2. Vogelstein, B. and Gillespie, D., Proc.Natl.Acad.Sci.,USA, vol.76,615,1979.
3. Smith, L.S., Lewis, T.L. and Matsui, S.M., Biotechniques, vol.18,972,1995.

Troubleshooting guide

| Sr. No. | Problem | Probable Cause | Solution |
|---------|--|---|---|
| 1. | Very less amount of DNA or no DNA obtained | More volumes of glass powder suspension used than desired | Using more volumes of Glass Powder Suspension could be detrimental to DNA yield and may result in elution of very less amount of DNA or no DNA. Hence, it is recommended to use volumes that correspond to the amount of DNA. |
| | | Glass powder settles at the bottom of the tube | The glass powder should not be allowed to settle at the bottom. Glass powder: DNA mixture should be mixed well every 2-3 minutes for proper binding. |

| | | | |
|----|--|--|---|
| | | Traces of residual Chaotropic Salt Solution | Prior to the elution of DNA, remove all the residual Chaotropic Salt Solution by washing with ethanol. This is important because if present in the final elution, it may lower the recovery of DNA or inhibit enzymes used in subsequent reactions. |
| | | Pellet is too dry | Do not vacuum dry the pellet for long time. Resuspend the pellet in TE or water and incubate at 55°C for 5 minutes. |
| | | Ethanol or water was not added in proper proportion to the Wash Buffer | The Wash Solution should be correctly prepared by the addition of ethanol and water as mentioned in preparation instructions. Store at -20°C after preparation. |
| | | The volume of elution buffer or water is insufficient | Repeat the elution step 2-3 times with new aliquots of TE or water. |
| | | Inappropriate elution buffer | DNA should be eluted efficiently in the presence of low salt buffer e.g. 10mM Tris-Cl, pH 8.5. |
| 2. | Agarose gel was not completely solubilized | Incorrect incubation temperature and time | The incubation temperature for solubilization of the gel should be 55°C. The incubation time can be increased till the gel completely dissolves. |
| | | Insufficient amount of Chaotropic Salt Solution added. | The amount of Chaotropic Salt Solution to be added should be 3 volumes. |
| 3. | Incomplete dissolution of DNA | Over drying of the pellet | Pellet should not be dried for more than 5 minutes. |
| | | Traces of ethanol left in the tube | Wash Buffer has to be completely removed or else traces of ethanol remaining in the tube may interfere with the enzymatic reactions. |

Safety Information

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.

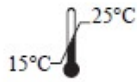
Disposal

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.

Please refer disclaimer Overleaf.



Storage temperature



Do not use if package is damaged



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