MB507 HiPurA® Plant Genomic DNA Miniprep Purification Kit

Kit Contents

Product Code	Reagents provided	MB507		
		20 Preps	50 Preps	250 Preps
DS0016	Lysis Buffer (PL)	10 ml	25 ml	125 ml
DS0054	Additive-I	68 mg	170 mg	850 mg
DS0017	Precipitation Buffer (PS)	3.2 ml	8 ml	40 ml
DS0018	Binding Buffer Concentrate (BB)	12 ml	30 ml	150 ml
DS0019	Wash Solution Concentrate (WSP)	12 ml	30 ml	150 ml
DS0040	Elution Buffer (ET) [10mM Tris-Cl, pH 8.5]	6 ml	15 ml	75 ml
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
DSCA02	HiShredder (in DBCA016 Collection tube)	20 nos	50 nos	250 nos
DBCA016	Collection Tube (Uncapped), Polypropylene (2.0 ml)	20 nos	50 nos	250 nos
DBCA017	Collection Tube, Polypropylene (2.0 ml)	40 nos	100 nos	2 X 250 nos

Intended Use

Recommended for isolation of DNA from Plant samples.

Introduction

HiPurA® Plant Genomic DNA Miniprep 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 genomic DNA is obtained from various species. The DNA obtained is compatible with downstream applications such as restriction endonuclease digestion, PCR and Southern blotting.

HiPurA® Plant Genomic DNA Miniprep Purification Kit

This kit simplifies isolation of DNA from fresh plant material with spin column procedure. The procedure is optimized for a maximum of 100 mg of wet-weight of the starting material. The sample (fresh) is cut and ground in liquid nitrogen along with Lysis Buffer (PL). Protein precipitation is followed by removal of other contaminants using HiShredder. The flow-through fraction is then mixed with a solution that enhances the binding of DNA to the column. The solution is then passed through HiElute Miniprep Spin Column (Capped) that is followed by washing steps to remove trace contaminants. High quality DNA is eluted in the Elution Buffer (ET) provided in the kit. Typical yield from 100mg of wet-weight sample is 5-40 µg (depending upon the type of plant used).

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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 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.

HiShredder (DSCA02)

For the plant genomic DNA isolation procedure, contaminants such as cell debris, salt precipitates are removed by centrifugation through a HiShredder. The HiShredder removes all cell debris and precipitates making the preparation of a clear lysate rapid and efficient. Preparation of the clear lysate is essential to prevent clogging of the HiElute Miniprep Spin Column (Capped) used in the subsequent steps.

Elution

The yield of genomic DNA depends on the sample type and the number of cells in the sample. 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 endonuclease digestion, Southern blotting applications etc.

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 260nm, 280nm, and 320nm using a quartz microcuvette. Absorbance readings at 260nm should fall between 0.1 and 1.0. The 320nm absorbance is used to correct for background absorbance. An absorbance of 1.0 at 260nm corresponds to approximately 5 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 260nm to absorbance at 280nm. DNA purified by HiPurA® Plant Genomic DNA Miniprep 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

- Small mortar and pestle
- Liquid nitrogen
- Chloroform:Isoamyl alcohol (24:1) (Product Code: MB115) (only for Alternative protocol)
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes) capable of upto ≈13,000 rpm
- Ethanol (96-100%)
- 95°C and 65°C water bath or heating block
- Molecular Biology Grade Water (Product Code: ML064)

Storage

Store the HiPurA® Plant Genomic DNA Miniprep Purification Kit 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 95°C or 65°C as required.
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 Binding Buffer Concentrate (BB) (DS0018) as follows:**

Number of Preps	Binding Buffer Concentrate (BB)	Ethanol (96-100 %)
20	12 ml	6 ml
50	30 ml	15 ml
250	150 ml	75 ml

5. **Dilute Wash Solution Concentrate (WSP) (DS0019) as follows:**

Number of Preps	Wash Solution Concentrate (WSP)	Ethanol (96-100 %)
20	12 ml	28 ml
50	30 ml	70 ml
250	150 ml	350 ml

6. Prechill the mortar and pestle at -20°C.
7. Dissolve 2.6 mg of Additive-I in 400μl of Lysis Buffer (PL) and heat the solution to 95°C before use (Only for Hard tissues).

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.

NOTE: It is preferable to use young plant parts especially leaves, needles (in case of pine, fir etc), since they contain more cells per weight and therefore result in higher yields. Also, young leaves and needles contain less polysaccharides and polyphenolics and are therefore easier to handle.
NOTE: Refer Alternative protocol for leaves/seeds rich in oil (e.g. - Eucalyptus leaves, Groundnut seeds, Castor seeds etc.), medicinal plants and dried leaves. We recommend the user to try the regular as well as alternative protocol for their plant tissue samples for optimum yield and purity.

Specimen Collection and Handling

For leaves/ flowers/ fruits/ stem
Collect plant tissue in a sterile container and freeze the sample at -20°C for short term storage or -80°C for long term storage.

For roots
Remove excess soil and collect plant tissue 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: leaves, flowers, fruits, stem and roots

Sample Preparation (Manual Disruption with mortar and pestle)

For Leaf Sample: Finely cut the leaf material before grinding. Midrib should be removed before cutting the leaves as midrib is a major source of carbohydrate contamination. Weigh 100 mg of the finely cut plant material and grind properly using a mortar and pestle in liquid nitrogen to a fine powder. Allow the liquid nitrogen to evaporate. DO NOT ALLOW THE SAMPLE TO THAW (keep samples on ice if needed). Proceed immediately to the DNA isolation protocol.

For Seeds: Grind about 100mg of soaked seeds in liquid nitrogen to a fine powder. Allow the liquid nitrogen to evaporate. DO NOT ALLOW THE SAMPLE TO THAW (keep samples on ice if needed). Proceed immediately to the DNA isolation protocol.

NOTE: Delay in DNA isolation after sample preparation will result in DNA degradation and yield loss.

A. Protocol for Hard and Soft tissues

For Hard tissues

1. To the ground material, immediately add 400 µl of Lysis Buffer (PL) (DS0016) containing Additive-I (DS0054) (preheated to 95°C) and mix thoroughly. [Do not grind the plant material after the addition of Lysis Buffer (PL), as it will cause shearing of DNA].

   NOTE: For the preparation of Lysis Buffer (PL) refer General Preparation Instructions.

2. Transfer the mixture to a capped 2.0 ml collection tube. Vortex vigorously.

3. Incubate the mixture for 10 minutes at 95°C, mix the contents 2-3 times by inverting the tube.

4. Add 130 µl of Precipitation Buffer (PS) (DS0017) to the lysate, mix and incubate for 5 minutes on ice.

5. **Load sample in HiShredder (DSCA02)**
   Add the entire sample to the HiShredder placed in a 2.0 ml collection tube (uncapped) and centrifuge for 5 minutes at a maximum speed (≈13,000 rpm) at room temperature (15-25°C). Transfer the flow-through fraction to a 2.0 ml collection tube (not provided) without disturbing the cell debris pellet.

6. Add 20 µl of RNase A Solution (20 mg/ml) (DS0003) to the above tube and incubate at room temperature (15-25°C) for 10 minutes. **(Continue with step 7: Binding)**.

<u>**For Soft tissues**</u>

1. To the ground material, immediately add 400 µl of Lysis Buffer (PL) (DS0016) and mix thoroughly. [Do not grind the plant material after the addition of Lysis Buffer (PL), as it will cause shearing of DNA].

2. Transfer the mixture to a capped 2.0 ml collection tube. Vortex vigorously.

3. Add 20 µl of RNase A Solution (20 mg/ml) (DS0003) to the above tube and incubate at room temperature (15-25°C) for 10 minutes.

4. Incubate the mixture for 10 minutes at 65°C, mix the contents 2-3 times by inverting the tube.

5. Add 130 µl of Precipitation Buffer (PS) (DS0017) to the lysate, mix and incubate for 5 minutes on ice.

6. **Load sample in HiShredder (DSCA02)**
   Add the entire sample to the HiShredder placed in a 2.0 ml collection tube (uncapped) and centrifuge for 5 minutes at a maximum speed (≈13,000 rpm) at room temperature (15-25°C). Transfer the flow-through fraction to a 2.0 ml collection tube (not provided) without disturbing the cell debris pellet. **(Continue with step 7: Binding)**.

7. **Binding**
   (Prepare the Binding Buffer as indicated in General Preparation Instructions)
   Add 1.5 volumes of diluted Binding Buffer (BB) (DS0018) to the lysate obtained from the above step and mix by pipetting.

   NOTE: E.g.: To 450 µl of lysate add 675 µl of diluted Binding Buffer (BB). The volume of buffer can be reduced accordingly if less lysate is obtained. A precipitate may form after the addition of Binding Buffer but this will not affect the DNA isolation procedure.

8. **Load lysate in HiElute Miniprep Spin Column (Capped) (DBCA03)**
   Add 650 µl of the lysate, including any precipitate, which may have formed, to the column placed in a 2.0 ml collection tube (uncapped). Centrifuge for 1 minute at 6000 x g (≈8000 rpm) at room temperature (15-25°C). Discard the flow-through.

9. Repeat the above step with the remaining sample. Discard the flow-through and reuse the 2.0 ml collection tube (uncapped).

10. **Wash**
    (Prepare the Wash Solution as indicated in General Preparation Instructions)
    Add 500 µl of diluted Wash Solution (WSP) (DS0019) and centrifuge for 1 minute at 6000 x g (≈8000 rpm) at room temperature (15-25°C).

NOTE: Discard the flow-through and reuse the 2.0 ml collection tube (uncapped).

11. Add another 500 µl of diluted Wash Solution (WSP) to the column and centrifuge for 2 minutes at a maximum speed (≈13,000 rpm) at room temperature (15-25°C). Discard the flow-through and reuse the same 2.0 ml collection tube (uncapped).

12. Centrifuge the tube with column for an additional 2 minutes at a maximum speed (≈13,000 rpm) at room temperature (15-25°C) to dry the membrane.

13. **DNA Elution**
    Place the column in a new 2.0 ml collection tube (uncapped) and pipette 100 µl of the Elution Buffer (ET) (DS0040) directly onto the column without spilling to the sides. Incubate for 5 minutes at room temperature (15-25°C). Centrifuge at ≥6,500 x g (≈10,000 rpm) at room temperature (15-25°C) for 1 minute to elute the DNA. Repeat the step again with another 100 µl of Elution Buffer (ET) for high yield of DNA.

    NOTE: DNA elution can also be performed in single step by the addition of 200 µl of Elution Buffer (ET) at a time (DNA yield would be low). Storing DNA in water may cause acid hydrolysis.

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

B. <u>Alternative Protocol for leaves/seeds rich in oil (e.g.- Eucalyptus leaves, Groundnut seeds, Castor seeds etc.), Medicinal plants and dried leaves</u>

NOTE: Leaves or seeds rich in oil will form a visible layer over the tissue homogenate during normal isolation of DNA and pose difficulty in recovery and further processing of supernatant. Moreover, even traces of lipids may hinder subsequent fingerprinting studies by making the samples hydrophobic and rendering them colloidal, adversely affecting the catalysis. This alternate protocol will remove oil from the tissues and provide good quality DNA which is suitable for downstream applications. It will also give excellent purity with medicinal plant tissue.

<u>Sample Preparation</u>

- **Oil seed samples (Groundnut seeds, Castor seeds)**
  For Groundnut, the seeds should be soaked in Molecular Biology Grade Water (ML064) or distilled water for 6 hours before starting the protocol. It is preferable to soak the castor seeds overnight before starting the protocol. Soaking increases the yield significantly. Proceed to **Sample Preparation (Manual Disruption with mortar and pestle)** for grinding of the seeds (given in regular DNA Isolation Protocol [above]).

- **Leaf samples**
  Process according to Sample Preparation protocol given in regular DNA isolation Protocol (above).

1. Add 400 µl of Lysis Buffer (PL) (DS0016) and 20 µl of RNase A stock solution (20 mg/ml) (DS0003) to a maximum of 100 mg of seeds/leaf that has been already disrupted by grinding [Refer to sample preparation (above) for grinding of seeds]. Vortex vigorously.

   NOTE: Do not mix Lysis Buffer (PL) and RNase A before use.

2. To the ground sample, add 400 µl of Chloroform: Isoamyl alcohol (24:1) (Product Code: MB115) and vortex to mix the contents of the tube. Centrifuge at 10,000 x g (≈13,000 rpm) for 2 minutes at room temperature (15-25°C). Carefully transfer the upper aqueous phase

to a capped 2.0 ml collection tube. Avoid the milky interphase containing carbohydrates, proteins and other inhibitors.

NOTE: This step will remove much of the polysaccharides and proteins from solution and improve spin column performance.

3. Incubate the upper aqueous phase for 10 minutes at 65°C, mix the contents of the tube 2-3 times by inverting the tube.

4. **Load lysate in HiShredder (DSCA02)**
   Add the lysate to the HiShredder placed in a 2.0 ml collection tube (uncapped) and centrifuge for 2 minutes at a maximum speed (≈13,000 rpm) at room temperature (15-25°C).
5. Transfer the flow-through fraction from step 4 to a 2.0 ml collection tube (not provided) without disturbing the cell debris pellet.

6. **Binding**
   (Prepare the Binding Buffer as indicated in General Preparation Instructions)
   Add 1.5 volumes of diluted Binding Buffer (BB) (DS0018) to the cleared lysate and mix by pipetting.

   NOTE: e.g.: To 450 µl of lysate add 675 µl of diluted Binding Buffer (BB). The volume of buffer can be reduced accordingly if less lysate is obtained. A precipitate may form after the addition of Binding Buffer but this will not affect the DNA isolation procedure.

7. **Load lysate in HiElute Miniprep Spin Column (Capped) (DBCA03)**
   Add 650 µl of the lysate from step 6, including any precipitate which may have formed into the HiElute Miniprep Spin Column (Capped) placed in a 2.0 ml collection tube (uncapped). Centrifuge for 1 minute at 6000 x g (≈8000 rpm) at room temperature (15-25°C). Discard the flow-through.

8. Repeat step 7 with the remaining sample. Discard the flow-through liquid and reuse the 2.0 ml collection tube (uncapped).

9. **Wash**
   (Prepare the Wash Solution as indicated in General Preparation Instructions)
   Add 500 µl of diluted Wash Solution (WSP) (DS0019) and centrifuge for 1 minute at 6000 x g (≈8000 rpm) at room temperature (15-25°C).

   NOTE: Discard the flow-through and reuse the 2.0 ml collection tube (uncapped) in step 10.

10. Add another 500 µl of diluted Wash Solution (WSP) to the column and centrifuge for 2 minutes at a maximum speed (≈13,000 rpm) at room temperature (15-25°C). Discard the flow-through liquid.

11. Centrifuge the tube with the empty column for an additional 2 minutes at a maximum speed (≈13,000 rpm) at room temperature (15-25°C) to dry the membrane.

12. **DNA Elution**
    Place the column in a new 2.0 ml collection tube (uncapped) and pipette 100 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 at room temperature (15-25°C) to elute the DNA. Repeat the step again with

another 100 µl of Elution Buffer (ET) for high yield of DNA. Transfer the eluate to a new capped 2.0 ml collection tube for DNA storage.

NOTE: To increase the elution efficiency, incubate the tube for 5 minutes at room temperature (15-25°C) after adding the Elution Buffer (ET), then centrifuge. DNA elution can also be performed in single step by the addition of 200 µl of Elution Buffer (ET) at a time (DNA yield would be low). Storing DNA in water can cause acid hydrolysis.

13. Transfer the eluate to a fresh capped 2 ml 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 hours) 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 (ET) 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® Plant Genomic DNA Miniprep Purification Kit is tested against predetermined specifications to ensure consistent product quality.

Quality Control

Type of Sample	DNA Yield	DNA Purity
Plant leaf sample (100 mg)	5-40 µg of DNA	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.	Clogged HiElute Miniprep Spin Column (Capped)	Carryover of the particulate material	Ensure that no particulate material is transferred following centrifugation through the HiShredder.
		Lysate is too viscous	The amount of starting material can be reduced or the amount of buffers PL and PS can be increased.
		Insufficient centrifugation	The g-force and the centrifugation time can be increased.

2.	Lower yields of DNA	Insufficient disruption of the plant tissue	Ensure that the plant material is disrupted in sufficient amounts of liquid nitrogen. It is very important that the disrupted tissue sample should not thaw before addition of Lysis Buffer (PL).
		Insufficient lysis	The amount of the starting material can be reduced or the amount of Buffers PL and PS can be increased.
		Incorrect binding	Ensure that the binding conditions are adjusted correctly by accurately determining the amount of lysate recovered.
		DNA still bound to the membrane	The volume of Elution Buffer (ET) or water can be increased up to 200 µl and tubes can be incubated at room temperature (15 – 25°C) for 5 minutes before centrifugation.
3.	Shearing of DNA	Precipitation of Lysis Buffer (PL)	Examine the solution for any kind of precipitation. If the solution 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.
4.	Darkly coloured membrane or green/yellow eluate after washing with Wash Solution	Insufficient washing of the membrane	After washing with the Wash Solution (WSP), an additional wash with 500 µl ethanol (96-100%) should be performed. Centrifuge for 2 minutes at a maximum speed (≈13,000 rpm) to dry the membrane.
		Amount of starting material is more than recommended	Reduce the amount of starting material for future preps.
5.	Poor performance of DNA in downstream experiments	Ethanol carryover	Ensure that after the second wash with Wash Solution (WSP), the column is centrifuged for 2 minutes at a maximum speed (≈13,000 rpm) to dry the membrane. Following the spin, remove the column carefully from the collection tube so that it does not come in contact with the flow through as this will result in carryover of ethanol.
		Salt carryover	Ensure that the Wash Solution (WSP) is at room temperature (15-25°C) before use.

Safety Information

HiPurA® Plant Genomic DNA Miniprep Purification Kit is for laboratory use only; not for drug, household or other uses. Take appropriate laboratory safety measures and wear gloves when handling. Avoid contact with skin, and use eye protection. In case of contact, wash with large amount of water. Seek medical attention. 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
LOT	Batch code		Temperature limit
	Date of manufacture (YYYY-MM)		Consult instructions for use
	Use-by date (YYYY-MM)	REF	Catalogue number

Identification No.: PIMB507
Rev. No.: 19
Date of Issue: 2025-05

Disclaimer :

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