MB602 HiPurA® Total RNA Miniprep Purification Kit

Kit Contents

Product Code	Reagents provided	MB602		
		20 Preps	50 preps	250 preps
DS0037	RNA Lysis Solution (HRL)	16 ml	40 ml	200 ml
DS0041	Prewash Solution (RW1)	18 ml	45 ml	225 ml
DS0012	Wash Solution Concentrate (WS)	8 ml	20 ml	100 ml
DS0042	Elution Solution (RNase- Free Water)	2.4 ml	6 ml	30 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

Introduction

HiPurA® Total RNA Miniprep Purification Kit provides a fast and easy method for purification of total RNA for Northern analysis, Poly A^+ RNA selection, Primer extension, RNase and S1 nuclease protection assays, RT-PCR, Differential display, Expression-array and expression-chip analysis and cDNA library construction. The RNA purification procedure using the miniprep spin columns comprises of three steps viz, adsorption of RNA to the membrane, removal of residual contaminants and elution of pure RNA. HiMedia's HiElute Miniprep Spin column (Capped) format allows rapid processing of multiple samples. The columns have a high binding capacity and high quality RNA is obtained from various species. The RNA obtained is compatible with various downstream applications as mentioned above.

HiPurA® Total RNA Miniprep Purification Kit

This kit simplifies isolation of RNA from animal cells, animal tissues and yeast, and for cleanup of RNA from crude enzymatic reactions (e.g. DNase digestion, Proteinase digestion, RNA ligation, and labeling reaction) with spin-column procedure. The lysis buffer provided in the kit helps in cell disruption and denaturation, samples are centrifuged through a HiShredder which removes insoluble material and reduces the viscosity of the lysate by disrupting viscous material. Ethanol is added to the cleared lysate, which promotes selective binding of RNA to the HiElute Miniprep Spin Column (Capped) membrane. After the initial binding of RNA, impurities like proteins, polysaccharides, low molecular weight metabolites and salts are removed by short washing steps. High quality RNA is finally eluted in the Elution Solution provided with the kit. A specialized high-salt buffer system allows upto 100 µg of total RNA longer than 200 bases to bind to the HiElute Miniprep Spin column (Capped) membrane.

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

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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 RNA. 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 RNA isolation techniques. RNA 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 Elution Solution provided with the kit. The ratio of 28S rRNA to 18S rRNA should be 2:1. The ribosomal RNAs should appear as sharp bands or peaks.

Elution

The yield of RNA depends on the sample type and the number of cells in the sample. A single elution with 30-50 µl of Elution Solution will provide sufficient RNA to carry out multiple amplification reaction.

Concentration, yield and purity of RNA

Spectrophotometric analysis and agarose gel electrophoresis will reveal the concentration and the purity of the RNA. Use Elution Solution 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 40 µg/ml of RNA. The A_{260}-A_{320}/A_{280}-A_{320} ratio should be 1.8 – 2.1. Purity is determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm. RNA purified by HiPurA® Total RNA Miniprep Purification Kit is free of protein and other contaminants that can inhibit PCR or other enzymatic reactions.

Concentration of RNA sample (µg/ml) = 40 x A_{260} x dilution factor.

Materials needed but not provided

- 30°C water bath - For Isolation of total RNA from Yeast
- Tabletop Microcentrifuge (with rotor for 2.0 ml tubes)
- RNase – free pipette tips (aerosol barrier recommended)
- Ethanol (96 – 100%)
- Ethanol (70%)
- 2- mercaptoethanol (ß -ME) (Product Code: MB041)
- Deoxyribonuclease I Solution (RNase-Free) and DNase Digest Buffer (procured from any standard company)
- Lyticase /zymolase (Product Code: MB099), 1 M Sorbitol and 0.1 M EDTA, pH 7.4 - For Isolation of total RNA from Yeast

Storage

HiPurA® Total RNA Miniprep Purification Kit can be stored at room temperature (15-25°C) for up to 18 months without showing any reduction in performance.

Precautions to be taken while handling RNA

Ribonucleases (RNases) are very stable and active enzymes that generally do not require cofactors to function. Since RNases are difficult to inactivate and even minute amounts are sufficient to destroy RNA, do not use any plasticware or glassware without first eliminating possible RNase contamination. Great care should be taken to avoid inadvertently introducing RNases into the RNA sample during or after the isolation procedure. In order to create and maintain an RNase-free environment, the following precautions must be taken during pretreatment and use of disposable and non- disposable vessels and solutions while working with RNA.

1. Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contamination from surface of the skin or from dusty laboratory equipment. Change gloves frequently and keep tubes closed whenever possible.

2. Use sterile, disposable plasticware and autoclavable pipettes reserved for RNA work to prevent cross-contamination with RNases from shared equipments.

3. Non-disposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1M NaOH, 1mM EDTA followed by RNase-free water. Alternatively, chloroform-resistant plasticware can be rinsed with chloroform to inactivate RNases.

4. Glassware used for RNA work should be cleaned with a detergent, thoroughly rinsed, and oven baked at 240ºC for four or more hours before use. Alternatively glassware can be treated with DEPC (Diethyl pyrocarbonate). Fill glassware with 0.1% DEPC (0.1% in water), allow to stand overnight at 37°C, and then autoclave or heat to 100°C for 15 min to eliminate residual DEPC.

5. Electrophoresis tanks should be cleaned with detergent solution (e.g., 0.5% SDS), thoroughly rinsed with RNase-free water, and then rinsed with ethanol and allowed to dry.

6. Solutions (water and other solutions) should be treated with 0.1% DEPC.

General Preparation Instructions

1. **ß -mercaptoethanol (ß -ME) must be added to RNA Lysis Solution (HRL) before use.**
   ß-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10µl ß-ME per 1ml Lysis Solution. Lysis Solution containing ß-ME can be stored at room temperature for up to 1 month.

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 Nuclease-free 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	8 ml	24 ml
50	20 ml	60 ml
250	100 ml	300 ml

5. **For Yeast protocol**

   Buffer for Enzymatic lysis:

Depending on the yeast strain and enzyme used, the amount of enzyme and composition of this buffer may vary. Please adhere to guidelines of enzyme supplier. However, in most cases Buffer Y1 can be used.

Buffer Y1 1 M Sorbitol
0.1M EDTA, pH 7.4

Just before use, add:
10 µL of ß –mercaptoethanol per 1 ml of Sorbitol buffer.

6. Preset the centrifuge at 4°C for Yeast RNA Isolation protocol.

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

A. <u>Protocol for Isolation of Total RNA from Animal Cells</u>

1. **Harvest the cells according to step a, b, or c**

a. Cells grown in suspension (do not use more than 1 x 10^7 cells):
Pellet up to 1 x 10^7 cells by centrifuging for 5 minutes at 300 x g (≈1500 rpm) in a Collection tube (not supplied). Discard the culture medium completely and continue with step 2 of Lysis reaction.

NOTE: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the binding of RNA to the HiElute Miniprep Spin Column (Capped) membrane resulting in lower RNA yield.

b. Cells grown in a monolayer (do not use more than 1 x 10^7 cells):
Direct lysis of the cells can be done in the cell-culture vessel (up to 10 cm diameter) or trypsinized and collected as a cell pellet prior to lysis. Cells grown in cell-culture flasks should always be trypsinized.

To trypsinize and collect cells:
Determine the number of cells. Aspirate the medium, and wash the cells with PBS. Aspirate the PBS, add 0.1–0.25% trypsin in PBS. After the cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer the cells to an RNase-free glass or polypropylene centrifuge tube (not supplied), and centrifuge at 300 x g for 5 minutes. Completely aspirate the supernatant, and proceed to step 2 of Lysis reaction.

NOTE: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the binding of RNA to the HiElute Miniprep Spin Column (Capped) membrane resulting in lower RNA yield.

c. To lyse cells directly in culture dish:

Determine the number of cells. Completely aspirate the cell-culture medium, and proceed immediately to step 2 of Lysis reaction.

NOTE: Incomplete removal of cell-culture medium will inhibit lysis and dilute the lysate, affecting the binding of RNA to the HiElute Miniprep Spin Column (Capped) membrane resulting in lower RNA yield.

2. **Lysis reaction**

a. For pelleted cells, loosen the cell pellet thoroughly by flicking the tube. Add the appropriate volume of RNA Lysis Solution (HRL) (DS0037) (see the table below). Vortex or pipet to mix, and continue with step 3.

NOTE: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced RNA yields. Ensure that ß -ME is added to RNA Lysis Solution (HRL) before use.

Number of pelleted cells	RNA Lysis Solution (HRL)
$<5x10^6$	350 µl
$5x10^6$ - $1x10^7$	600 µl

b. For Cells grown in a monolayer, add appropriate volume of RNA Lysis Solution (HRL) (see the table below) to the cell culture dish. Collect cell lysate into a collection tube (not supplied). Vortex or pipet to mix and ensure that no cell clumps are visible before proceeding to step 3.

NOTE: Regardless of the cell number, use the Lysis Solution volumes indicated to completely cover the surface of the dish. Ensure that ß -ME is added to RNA Lysis Solution (HRL) before use.

Dish diameter	RNA Lysis Solution (HRL)
< 6 cm	350 µl
6-10 cm	600 µl

3. **Homogenize the lysate**

If processing ≤1 x 10^5 cells, homogenize by vortexing for 1 minute. After homogenization, proceed to step 4.

NOTE: Incomplete homogenization leads to significantly reduced RNA yields and can cause clogging of the HiElute Miniprep Spin Column (Capped). Homogenization with a HiShredder generally results in higher RNA yields.

4. Pipet the lysate directly into a HiShredder (DSCA02) placed in a 2 ml uncapped collection tube, and centrifuge for 2 minutes at 13,000 X g (14,000 rpm). Transfer the supernatant to a new collection tube and Continue with binding**.**

B. <u>Protocol for Isolation of Total RNA from Animal Tissues</u>

1. **Fresh or frozen (up to 30 mg) tissue**.

Immediately place the weighed (fresh or frozen) tissue in liquid nitrogen, and grind thoroughly with a mortar and pestle. Allow the liquid nitrogen to evaporate. Do not allow the samples to thaw. Proceed with the lysis.

2. **Lysis reaction**

Add appropriate volume of RNA Lysis Solution (HRL) (DS0037) (see the table below). Mix properly and transfer it to an RNase free 2.0 ml capped collection tube. Vortex or pipet to mix.

Amount of starting material	RNA Lysis Solution (HRL)
<20 mg	350 µl
20 mg to 30 mg	600 µl

NOTE: Incomplete loosening of the cell pellet may lead to inefficient lysis and reduced RNA yields. Ensure that ß -ME is added to RNA Lysis Solution (HRL) before use.

3. Pipet the lysate directly into a HiShredder (DSCA02) placed in a 2 ml uncapped collection tube, and centrifuge for 2 min at full speed. Transfer the supernatant to a new collection tube and Continue with binding**.**

C. <u>Protocol for Isolation of Total RNA from Yeast</u>

Important notes before starting

For RNA isolation from Yeast, cells should be harvested in log-phase growth. Use only freshly harvested cells for the protocol.

The Yeast culture should be harvested at 4°C. All subsequent steps of the protocol should be performed at room temperature (15-25°C).

Procedure

1. **Harvest and Resuspend the Yeast cells**
   Harvest yeast cells by centrifugation at 1,000 X g for 5 minutes at 4°C. (Do not use more than $5x10^7$ yeast cells). Remove the culture medium and discard.

2. Resuspend the cells in 600 µl of freshly prepared Buffer Y1. Add 50U of zymolyase or lyticase and incubate at 30°C for 30 minutes with gentle shaking to generate spheroplasts. **(Prepare Buffer Y1 as indicated in General Preparation Instructions)**

3. Centrifuge for 5 minutes at 6500 x g (10,000 rpm) to pellet spheroplasts. Carefully remove and discard the supernatant.

4. **Lysis reaction**
   Add 350 µl of RNA Lysis Solution (HRL) (DS0037) to the pellet. Vortex or pipet to mix.

   NOTE: If insoluble material is visible, centrifuge for 2 minutes at maximum speed, and use only the supernatant in the subsequent steps. Ensure that ß -ME is added to RNA Lysis Solution (HRL) before use.

5. Pipet the lysate directly into a HiShredder (DSCA02) placed in a 2 ml uncapped collection tube, and centrifuge for 2 minutes at 13,000 X g (14,000 rpm). Transfer the supernatant to a new collection tube and Continue with binding**.**

D. <u>Protocol for RNA Cleanup</u>

Important notes before starting

A maximum of 100 µg RNA can be used in the RNA cleanup protocol.

Procedure

1. Adjust sample to a volume of 100 µl with RNase-free water. Add 350 µl of RNA Lysis Solution (HRL) (DS0037) and mix thoroughly.

   NOTE: Ensure that ß -ME is added to RNA Lysis Solution (HRL) before use.

2. **Prepare for binding:**
   Add 250 µl of ethanol (96-100%) to the diluted RNA, and mix thoroughly by pipetting. Do not centrifuge. Continue without delay with column step (below) followed by washing

with Wash Solution (WS) (DS0012) upto elution steps (below) [Skip the wash step with Prewash Solution (RW1) (DS0041) in RNA cleanup protocol].

RNA ISOLATION FROM ANIMAL CELLS, ANIMAL TISSUES, YEAST AND FOR RNA CLEANUP

- **Prepare for binding:**
  Add 1 volume of 70% ethanol to the homogenized lysate, and mix well by pipetting. Do not centrifuge.

  NOTE: A precipitate may be visible after addition of ethanol. This does not affect the procedure.

- **Load Lysate in HiElute Miniprep Spin Column (Capped) [DBCA03]**
  Apply sample including any precipitate that may have formed, on the HiElute Miniprep Spin Column (Capped). Close the tube gently, and centrifuge for 15 seconds at ≥8000 x g (≥10,000 rpm). Discard the flow-through.

  NOTE: If the volume exceeds 700 µl, load aliquots successively onto the HiElute Miniprep Spin column and centrifuge as above. Discard the flow-through after each centrifugation step.

  Optional: On Column DNase digestion
  Generally, DNase digestion is not required since the solutions of this kit efficiently remove most of the DNA without DNase treatment. However, further DNase treatment may be necessary for certain RNA applications that are sensitive to small amounts of DNA (e.g.TaqMan RT-PCR analysis with a low abundant target). DNA can also be removed by DNase digestion.

- Carryout lysis, homogenization, and loading onto the HiElute Miniprep Spin Column (Capped) as indicated above. Instead of continuing with the Prewash Solution (RW1), follow steps below.
- Pipet 350 µl of Prewash Solution (RW1) (DS0041) into the HiElute Miniprep Spin column (Capped), and centrifuge for 15 sec at ≥8000 x g (≥10,000 rpm). Discard the flow through and reuse the collection tube in step 6c.
- Add 10 µl of DNase I Solution to 70 µl of DNase Digest Buffer. Mix by inversion. Do not vortex.
- Add 80 µl of DNase I/Digest Buffer mixture directly onto the HiElute Miniprep Spin Column (Capped). Incubate at room temperature for 15 minutes.
- Pipet 350 µl of Prewash Solution (RW1) into the HiElute Miniprep Spin column , and centrifuge for 15 seconds at centrifuge at ≥8000 x g (≥10,000 rpm). Discard the flow-through and continue with the **Wash Step**.

Or

Alternatively, proceed with RNA isolation protocol without DNase I treatment.

- **Prewash**
  Add 700 µl of Prewash Solution (RW1) to the HiElute Miniprep Spin Column (Capped) centrifuge at ≥8000 x g (≥10,000 rpm) for 1 minute. Discard the flow-through. Reuse the collection tube.

- **Wash**

Pipet 500 µl of Wash Solution (WS) (DS0012). Close the tube gently, and centrifuge for 1 minute at ≥8000 x g (≥10,000 rpm) to wash the column. Discard the flow-through.

NOTE: Wash Solution (WS) is supplied as a concentrate. Ensure that ethanol is added to Wash Solution Concentrate (WS).

- Add another 500 µl of Wash Solution (WS) to the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 2 minutes at ≥8000 x g (≥10,000 rpm) to dry the membrane. Continue directly with elution step or to eliminate any chance of possible Wash Solution carryover, perform the next step.

  Optional: Place the column in a new 2 ml collection tube (not supplied), and discard the old collection tube with the flow-through. Centrifuge at 13,000 X g (14,000 rpm) for 1 minute.

- **RNA Elution**
  Transfer the HiElute Miniprep Spin column (Capped) to a new 2 ml capped collection tube. Pipet 30-50 µl Elution Solution (RNase-Free Water) directly onto the HiElute Miniprep Spin column (Capped). Close the tube gently, and centrifuge for 1 minute at ≥8000 x g (≥10,000 rpm) to elute.

- If the expected RNA yield is > 30 µg, repeat the elution step as described with a second volume of RNase-Free Water. Elute into the same collection tube.

- Transfer the eluate to a fresh capped 2ml collection tube for longer RNA storage.

  NOTE: To obtain a higher total RNA concentration, this second elution step may be performed by using the first eluate. The yield will be 15-30% less than the yield obtained using a second volume of RNase-free water, but the final concentration will be higher.

  Storage of the eluate with purified RNA: The eluate contains pure RNA, recommended to be stored at lower temperature (-80°C). Avoid repeated freezing and thawing of the sample which may cause denaturing of RNA.

References:

1. Sambrook, J., *et al.* Molecular Cloning: A laboratory Manual, 2nd ed. (Cold Spring Harbor Laboratory Press, Plainview, NY, 1989; pp. 7.3-7.5)
2. Farrell, Robert E., Jr.; RNA Methodologies; 2nd Edition; Academic Press: NY, 1998; pp. 37-53(Cat. No**.** Z350354)

Precautions

Read the procedure carefully before starting the experiment.

Performance and Evaluation

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

Quality Control

Type of Sample	RNA Yield	RNA Purity
CHO cells	upto 100 µg	1.8-2.1

Trouble shooting Guide:

Sr. No.	Problem	Possible Cause	Solution
1.	Clogged HiElute Miniprep Spin Column (Capped)	Too much starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material (see protocols).
		Tissues: Centrifugation before adding ethanol not performed	Centrifuge the lysate before adding ethanol and use only this supernatant in subsequent steps. Pellets contain cell debris can clog the HiElute Miniprep Spin Column (Capped).
		Centrifugation temperature is too low	The centrifugation temperature should be 20 – 25°C. Some centrifuges may cool to below 20°C even when set at 20°C. This can cause formation of precipitates that can clog the column. If this happens, set the centrifugation temperature to 25°C. Warm the ethanol containing lysate to 37°C before transferring it to the column.
2.	Low RNA Yield	Too much of starting material	In subsequent preparations, reduce the amount of starting material. It is essential to use the correct amount of starting material (see protocols).
		RNA still bound to HiElute Miniprep Spin Column	Repeat RNA elution, but incubate the column for 10 minutes at room temperature with Elution solution (RNase free water) before centrifuging.
		Ethanol carryover	During the second wash with Wash Solution (WS) be sure to centrifuge at ≥8000 x g (≥10, 000 rpm) for 2 minutes to dry the column. After centrifugation, carefully remove the column from the collection tube so that the column does not contact the flow through otherwise carryover of ethanol will occur. To eliminate any chance of possible ethanol, centrifuge the column for another minute at full speed.
		Incomplete removal of cell culture medium.	When processing cultured cells ensure complete removal of the cell culture medium after harvesting cells

3.	Low A_{260} / A_{280}	Water has been used to dilute RNA for A_{260} / A_{280} measurement.	Use 10 mM Tris – Cl, pH 7.5, not RNase free water to dilute the sample before measuring purity.
4.	DNA contamination in downstream experiments	No incubation with Prewash Solution (RW1)	In subsequent preparations, incubate the HiElute Miniprep Spin Column (Capped) for 5 minutes at room temperature after the addition of Prewash Solution (RW1) before centrifuging.
		No DNase treatment	Follow the optional on-column DNase digestion step.
5.	RNA does not perform well in downstream experiments	Ethanol carryover	During the second Wash using Wash Solution (WS), be sure to dry the HiElute Miniprep Spin Column membrane by centrifugation at ≥8000 x g (≥10,000 rpm) for 2 minutes to dry the membrane. Following the centrifugation, remove the HiElute Miniprep Spin Column from the collection tube carefully so the column does not contact the flow-through as this will result in carryover of ethanol.

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.

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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