ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Quick-RNA™ Miniprep Plus Kit

Catalog Nos. **R1057T, R1057,** & **R1058**

Highlights

- Purify high-quality total RNA (including small/micro RNAs) from any cells, tissues, blood and biological fluids.
- *DNA-free* RNA is ready for use in any downstream application. *DNase I included.*

Contents

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Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product please contact us.

Integrity of kit components is guaranteed for up to one year from date of purchase Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

For assistance, contact us at tech@zymoresearch.com.

Product Contents

***Quick*-RNA™ Miniprep Plus Kit** (Kit Size)	**R1057** (50 Preps.)	**R1058** (200 Preps.)	**R1057T** (10 Preps.)
RNA Lysis Buffer	50 ml	2x 100 ml	10 ml
RNA Prep Buffer	25 ml	100 ml	5 ml
RNA Wash Buffer[1] (concentrate)	24 ml	2x 48 ml	16 ml
DNase/RNase-Free Water	6 ml	30 ml	1 ml
DNase I[2] (lyophilized)	1	4	1
DNA Digestion Buffer	4 ml	16 ml	0.8 ml
DNA/RNA Shield™ (2X concentrate)	25 ml	125 ml	5 ml
PK Digestion Buffer	5 ml	20 ml	1 ml
Proteinase K[3] & Storage Buffer	20 mg	4x 20 mg	5 mg
Spin-Away™ Filters	50	200	10
Zymo-Spin™ IIICG Columns	50	200	10
Collection Tubes	100	400	20
Instruction Manual	1	1	1

Storage Temperature - Store all kit components (*i.e.,* buffers, columns) at room temperature.

[1] Before starting, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R1057) or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate (R1058). RNA Wash Buffer included with R1057T is supplied ready-to-use and does not require the addition of ethanol prior to use.

[2] Prior to use, reconstitute the lyophilized **DNase** I as indicated on the vial prior to use. Store aliquots at -20ºC.

[3] Prior to use, add 260 µl **Proteinase K Storage Buffer** to the 5 mg lyophilized **Proteinase K** (R1057T) or 1040 µl **Proteinase K Storage Buffer** to the 20 mg lyophilized **Proteinase K** (R1057, R1058) . Vortex to dissolve. Store at -20ºC.

Specifications

- **Sample Sources** – Any cells (animal, blood cells *etc.*), all tissues (tough-to-lyse, FFPE, *etc.*), blood, biological fluids, and enzymatic reactions (e.g., DNase I), samples in DNA/RNA Shield™, TRIzol® or RNA*later*™.
- **Sample Preservation** – **DNA/RNA Shield™** lyses cells, inactivates nucleases and infectious agents and is ideal for safe sample storage and transport at ambient temperatures (page 7).
- **RNA Size** – RNAs ≥17 nucleotides.
- **RNA Purity** – A_{260}/A_{280} >1.8, A_{260}/A_{230} >1.8. DNase I provided for complete removal of DNA.
- **RNA Recovery** – The RNA binding capacity of the **Zymo-Spin™ IIICG Column** is ~100 µg.
- **RNA Storage** – RNA eluted with **DNase/RNase-Free Water** (provided) can be stored at ≤70ºC. The addition of RNase inhibitors in highly recommended for prolonged storage.
- **Equipment Needed** – Microcentrifuge, vortex, and heat block (55°C), water bath or incubator.

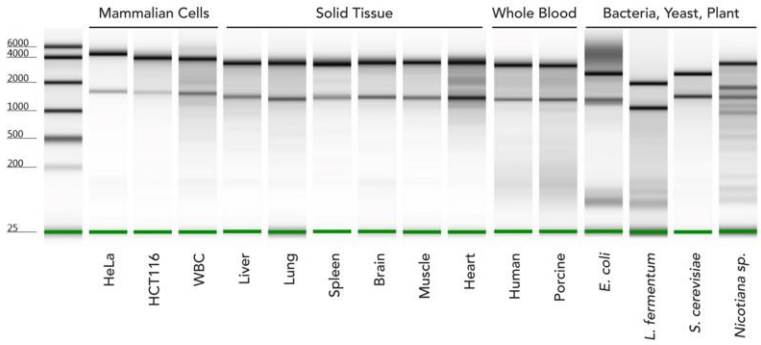
Product Description

The ***Quick*-RNA™ Miniprep Plus Kit** combines ***Quick*-RNA™** technology with the addition of **DNA/RNA Shield™**, a unique preservation and lysis technology, and **Proteinase K** to enable easy, reliable, and rapid isolation of RNA from any biological sample including any cells, all tissues, blood, and other biological fluids. The procedure uses *Zymo-Spin®* column technology that results in high-quality total RNA *(including small RNAs 17-200 nt)* that is *DNA-free* and is ready for RT-PCR, hybridization, sequencing, *etc*.

For **Assistance**, please contact Zymo Research Technical Support at 1-888-882-9682 or e-mail tech@zymoresearch.com.

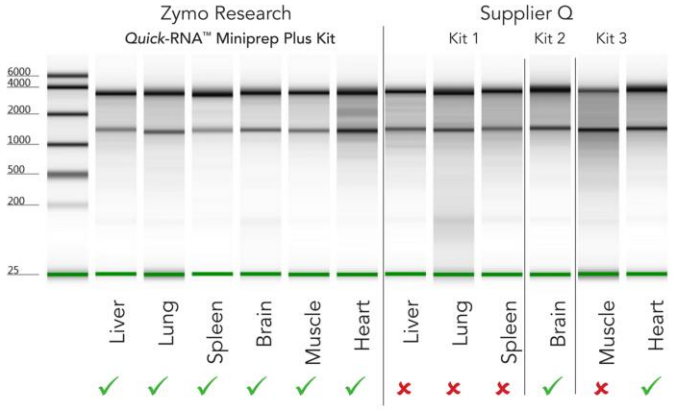
Universal

The ***Quick*-RNA™ Miniprep Plus Kit** is universal and accommodates any sample input including cells, tissue, blood *etc.*



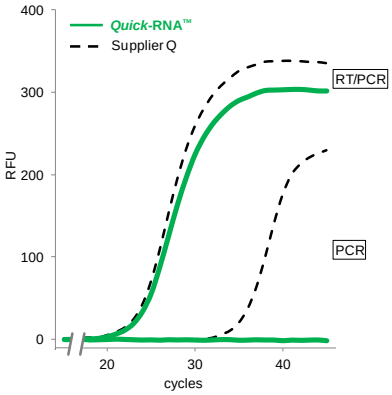
High Quality RNA

High quality RNA is consistently isolated with the ***Quick*-RNA™ Miniprep Plus Kit** (left), not with kits as recommended by Supplier Q (right). Quality assessed by Agilent 2200 TapeStation; red = low quality**.**



DNA-free RNA

RNA isolated with the ***Quick*-RNA™ Miniprep Plus Kit** is DNA-free. Samples isolated with Supplier Q's kit are provided for comparison. Total RNA was isolated from 10^6 human epithelial cells (with in-column DNase treatment for both kits). Each amplification curve represents an average of three independent isolation experiments.



	Quick-RNA™	Supplier Q
Small RNA (≥17 nt) recovery	Yes	No
DNase I included	Yes	No
gDNA removal column included	Yes	No
Proteinase K	Yes*	No
DNA/RNA Shield™ (for sample storage)	Yes*	No

*Quick-RNA™ MiniPrep Plus

Purification Guide

Sample Input	Start on
Cells (mammalian, blood cells)	**Page 4**
Tissue (soft, fibrous, lipid, FFPE)	
Whole Blood (biological liquids)	**Page 5**
Tough-to-Lyse (bacteria, yeast, plant)	
Reaction Clean-Up (in vitro processed RNA)	
Preserved Samples (DNA/RNA Shield™, TRIzol®, RNAlater™)	

RNA Yield and Kit Capacity

Sample Input	Average RNA Yield	Kit Capacity
Cells	10 µg (per 10^6 cells)	Up to 10^7
HeLa	15 µg	
High Yield Tissue[1] (mouse)	≥30 µg (per 10 mg)	Up to 20 mg
Spleen	30-50 µg	
Liver	40-60 µg	
Low Yield Tissue[1] (mouse)	≤30 µg (per 10 mg)	Up to 50 mg
Brain, Heart	5-15 µg	
Muscle	5-20 µg	
Lung	10-20 µg	
Intestine	10-30 µg	
Kidney	20-30 µg	
Whole Blood[2]	(per 1 ml)	Up to 1 ml
Porcine	10-20 µg	
Human	2-10 µg	

Notes:

[1] Yield from tissue samples can vary due to other factors such as organism type, physiological state, and growth conditions.

[2] Yield from blood samples can vary based upon the donor, age, and/or health conditions.

Reagent Preparation

- ✓ Before starting, add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **RNA Wash Buffer** concentrate (R1057) or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate (R1058).
- ✓ Add 275 µl **DNase/RNase-Free Water** per vial to reconstitute the lyophilized **DNase I** at 1 U/µl. Mix by gentle inversion. Store frozen aliquots at -20ºC.
- ✓ Add 260 µl **Proteinase K Storage Buffer** to the 5 mg lyophilized **Proteinase K** (R1057T) or 1040 µl **Proteinase K Storage Buffer** to the 20 mg lyophilized **Proteinase K** (R1057, R1058). Vortex to dissolve. Store at -20ºC.

Ensure the RNA isolation procedure is performed in an RNase-free environment.

The lyophilized **Proteinase K** and **DNase I** are stable as shipped.

Protocols

The RNA isolation consists of two steps: (I) Sample Preparation & (II) RNA Purification.

Sample Preparation

All centrifugation steps should be performed at 10,000 - 16,000 *x g* for 30 seconds unless specified.
All steps should be performed at room temperature (20-30ºC) unless specified.

Cells

Pellet[1] mammalian cells by centrifugation (≤500 *x g* for 1 minute), remove the supernatant and resuspend the cell pellet in **RNA Lysis Buffer**[2] (see table below). Proceed to page 6.

Mammalian Cells	Add RNA Lysis Buffer
≤5x10^6	300 µl
5x10^6 - 10^7	≥600 µl

Solid Tissue & Blood Cells (PBMCs, WBCs)

1. Add **DNA/RNA Shield**™ (1X)[3] to a solid tissue sample (see table below). Tissue samples can be mechanically homogenized for optimal extraction efficiency.

   For blood cells, buffy coat and pelleted PAXgene™ samples, resuspend in **DNA/RNA Shield**™ (1X).

Animal Tissue	Blood Cells	Add DNA/RNA Shield™ (1X)
≤50 mg	≤5 ml blood	≥300 µl[4]

2. For every 300 µl of sample, add 30 µl **PK Digestion Buffer** and 15 µl **Proteinase K**.
3. Mix and then incubate at 55ºC until tissue dissolves or up to 5 hours.[5]

Sample	Incubation Time
Non-homogenized	2-5 hours
Homogenized tissue	30 minutes
Blood cells (or PAXgene™ pellet)	30 minutes

4. After incubation, vortex sample and then centrifuge at max speed for 2 minutes to pellet debris. Transfer the aqueous supernatant into an RNase-free tube (not provided).
5. Add an equal volume of **RNA Lysis Buffer** and mix well. Proceed to page 6.

Notes:

[1] Cells in suspension and other liquids may be processed directly by adding 4 volumes of **RNA Lysis Buffer** and mixing. Proceed to page 6.

[2] Cell samples homogenized in **RNA Lysis Buffer** can be stored frozen for processing at a later time.

[3] To prepare 1X solution, mix equal amounts of the supplied 2X concentrate with nuclease-free water (not provided).

FFPE tissue (page 7).

[4] Solid tissue samples should be completely submerged in **DNA/RNA Shield**™, add as needed.

[5] Optimal incubation times may vary with tissue type and homogenization method.

Notes:

[1] Up to 200 µl can be processed without having to reload the spin column.

With reloading, up to 3 ml blood per prep can be processed.

**For bacterial, fungal, fecal, and soil samples, use the 0.5 mm beads (S6002).*

**For plant/seed, solid tissues, and insect samples, use the 2.0 mm beads (S6003).*

[2] Some Gram-negative bacteria (*e.g. E. coli*) may not require the **BashingBead™** system and can be lysed directly in **DNA/RNA Shield™**.

Sample preservation with DNA/RNA Shield™ (page 7).

[3] Compatible with: TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ and all other *acid-guanidinium-phenol* reagents.

Sample Preparation (continued)

Whole Blood (Mammalian)

1. Add 200 µl[1] **DNA/RNA Shield™** (2X concentrate) directly to each 200 µl of fresh/frozen blood sample and mix thoroughly.
2. For every 400 µl of reagent/blood mixture, add 8 µl **Proteinase K** and mix thoroughly. Incubate at room temperature (20-30ºC) for 30 minutes.
3. Add an equal volume of isopropanol and mix by vortex. Proceed to page 6, step 3.

Tough-to-Lyse Samples (Bacteria, Yeast, Plant, *etc.*)

Tough-to-lyse samples (including gram-positive bacteria) should be mechanically homogenized (*i.e.* ZR BashingBead™ Lysis Tubes*) directly in **DNA/RNA Shield™** (1X; mix equal amounts of the supplied 2X concentrate with nuclease-free water (not supplied)). Centrifuge and transfer the supernatant into an RNase-free tube. Add an equal volume of **RNA Lysis Buffer** and mix well. Proceed to page 6.

Bacteria[2]	Yeast	Plant/Seed	Add DNA/RNA Shield™ (1X)
≤5x10^7	≤5x10^6	-	400 µl
5x10^7 - 10^9	5x10^6 - 5x10^7	≤200 mg	≥800 µl

For the removal of (RT)PCR inhibitors (fecal, soil, plant samples), use the OneStep™ PCR Inhibitor Removal Kit (D6030).

Reaction Clean-Up

DNase-treated RNA, labeling, *in vitro* transcription and other enzymatic reactions can be processed directly. First, add 100 µl **RNA Lysis Buffer** to a 50 µl sample and mix, then add 1 volume 95-100% ethanol (150 µl ethanol to 150 µl mixture) and mix again. Proceed to page 6, step 3.

Samples in DNA/RNA Shield™

Bring samples homogenized and stored in **DNA/RNA Shield™** (1X) to room temperature (20-30ºC). Add 1 volume of **RNA Lysis Buffer** (1:1) and mix well. Proceed to page 6.

Samples in TRIzol® or similar

Following TRIzol®/chloroform or similar[3] extraction, carefully transfer the upper aqueous phase into an RNase-free tube (not provided). For each volume of the aqueous phase, add an equal volume of ethanol (95%-100%) and mix well. Proceed to page 6, step 3.

Samples in RNA*later*™

To process cells or liquids in RNA*later*™ (without reagent removal): Add 1 volume of RNase-free water or PBS to the sample (1:1). Then add 4 volumes **RNA Lysis Buffer** (4:1) and mix. Proceed to page 6.

Note: Alternatively, remove the RNA*later*™, then proceed with Sample Preparation according to the sample type.

RNA Purification

All centrifugation steps should be performed at 10,000 – 16,000 x *g* for 30 seconds unless specified.

1. Transfer the sample lysed in **RNA Lysis Buffer** into a **Spin-Away™ Filter**[1] (**yellow**) in a **Collection Tube** and centrifuge to remove the majority of gDNA.

   Save the flow-through.

2. Add 1 volume ethanol (95-100%) to the sample flow-through and mix well.

   Note: Alternatively, to isolate RNAs ≥200 nt, add ½ volume ethanol (95-100%) to the sample flow-through.

3. Transfer the mixture to a **Zymo-Spin™ IIICG Column**[1] (**green**) in a **Collection Tube** and centrifuge[2]. Discard the flow-through.

   Recommended: **DNase I** treatment (in-column)[3]

   (D1) Wash the column with 400 µl **RNA Wash Buffer** and centrifuge. Discard the flow-through.
   (D2) In an RNase-free tube, add 5 µl **DNase I** (1 U/µl)*, 75 µl **DNA Digestion Buffer** and mix. Add the mix directly to the column matrix.
   (D3) Incubate the column at room temperature (20-30ºC) for 15 minutes. Proceed to step 4.

4. Add 400 µl **RNA Prep Buffer** to the column and centrifuge. Discard the flow-through.

5. Add 700 µl **RNA Wash Buffer** to the column and centrifuge. Discard the flow-through.

6. Add 400 µl **RNA Wash Buffer** and centrifuge the column for 2 minutes to ensure complete removal of the wash buffer. Transfer the column carefully into an RNase-free tube (not provided).

7. Add 100 µl **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

   Alternatively, for highly concentrated RNA use ≥50 µl elution.

   The eluted RNA can be used immediately or stored at ≤-70ºC.

Notes:

[1] To process samples >700 µl, **Zymo-Spin™** columns may be reloaded.

[2] For processing large volumes, a vacuum manifold can be used for this step. Following the vacuum step, centrifuge the column to remove residual liquid.

[3] Prior to use, reconstitute the lyophilized **DNase I** as indicated on the vial. Store frozen aliquots.

** Unit definition - one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 A_{260} units/min/ml of reaction mixture at 25°C.*

Appendices

Sample Preservation in DNA/RNA Shield™

DNA/RNA Shield™ effectively lyses cells, inactivates nucleases and infectious agents and is ideal for sample storage/transport at ambient temperatures prior to nucleic acid purification.

Liquid samples: Mix an equal volume DNA/RNA Shield™ (2X concentrate) and sample.
Solid samples: Submerge sample (not to exceed 10% (v/v or w/v) in DNA/RNA Shield (1X).

Mix well/homogenize sample prior to storage.

Samples in DNA/RNA Shield™ can be stored at ambient temperature (4-25ºC) for a month, 3 days at 37ºC or long term (>1 year) at -20ºC or below.

Purification of Small and Large RNAs into Separate Fractions

This procedure is compatible with animal cell inputs (up to 10^6) or previously isolated RNA only.

All centrifugation steps should be performed between 10,000-16,000 x *g* for 30 seconds unless specified.
This protocol requires two columns (per prep).

1. Mix an equal volume of **RNA Lysis Buffer** and ethanol (95-100%).
   Example: Mix 50 µl buffer and 50 µl ethanol.
2. Add 2 volumes of the buffer/ethanol to an RNA sample[1] or 300 µl buffer/ethanol to a cell pellet, and mix well.
   Example: Mix 100 µl buffer/ethanol and 50 µl sample.
3. Transfer the mixture[2] to the **Zymo-Spin**™ **Column** in a **Collection Tube** and centrifuge. **Save the flow-through!**

	Column: RNAs >200 nt	**Flow-through**: RNAs 17-200 nt
4.	Continue to step 5.	Add 1 volume ethanol and mix (e.g., 150 µl ethanol to 150 µl flow-through). Transfer the mixture to a new column and centrifuge for 30 seconds. Discard the flow-through.

5. Proceed with RNA Purification (page 6, step 4).

FFPE Tissue: Deparaffinization

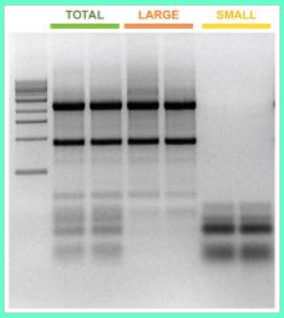
1. Remove (trim) as much excess paraffin from the sample as possible.
2. Transfer sample to a microcentrifuge tube (not provided).
3. Add 1 ml xylene and mix well. Then centrifuge for 1 minute and remove xylene.
4. Add 1 ml ethanol (95-100%) and mix well. Then centrifuge for 1 minute and remove ethanol. Repeat this step.
5. Dry samples by vacuum centrifugation (Speed-Vac) or by incubating uncapped tubes at ≤37 °C. It may take up to 40 minutes for a sample to air dry.
6. To purify RNA, follow Sample Preparation for tissue (page 4) and RNA Purification (page 6).

Notes:

[1] Adjust the sample volume to 50 µl (minimum).

[2] To process samples >700 µl, **Zymo-Spin**™ columns may be reloaded.

Total RNA (>17 nt), large (>200 nt) or small RNAs (17-200 nt) are effectively partitioned and purified with the ***Quick*-RNA**™ kit.



Troubleshooting:

For **Technical Assistance**, please call 1-888-882-9682 or e-mail tech@zymoresearch.com.

Problem	Possible Causes and Suggested Solutions
Sample Degradation	Sample Preservation • Immediately submerge tissue samples in **DNA/RNA Shield™** to ensure nucleic acid stability. • Using frozen whole blood (EDTA, citrate) samples can cause cell damage from membrane shock and shearing, resulting in degradation. For best results, store fresh whole blood in DNA/RNA Shield™ (2X concentrate). Alternatively, add DNA/RNA Shield™ to frozen blood samples prior to thawing.
Low Yield	Sample Input • For "high yield" samples, if the lysate is extremely viscous or did not pass through the column, use less input material. Too much input can cause cellular debris to overload the column and result in compromised RNA recovery. • For "low yield" samples (e.g., muscle), using larger inputs will increase yields (≤ 50 mg). Refer to the "RNA Yield" table (page 3). Incomplete Lysis/Digestion • Proteinase K incubation times may be extended depending on the type of sample (e.g., fibrous tissues). Elution • Reload the eluate onto the column and centrifuge again. Alternatively, heat the nuclease-free water to 95ºC before use.
Residual DNA	DNA removal • Perform *in-column* DNase I treatment to remove DNA. For "high-yield" samples, do not overload the Spin-Away Filter™ (titrate the input if necessary).
Low $A_{260/230\ nm}$	Sample Handling • There may be ethanol and/or salt contamination. Carefully remove the column from the collection tube so that there is no liquid contact. Blot emptied collection tubes with a tissue or towel to minimize liquid retention. • Make sure lysate has passed completely through matrix in the column before proceeding to wash steps. This may require centrifuging at a higher speed and/or longer time.

Ordering Information

Product Description	Input	Binding	Catalog No.	Kit Size
***Quick*-RNA™ Microprep Kit**	~1-10^6 cells	~10 µg	R1050 R1051	50 Preps. 200 Preps.
***Quick*-RNA™ Miniprep Kit**	~10^2-10^7 cells	~100 µg	R1054 R1055	50 Preps. 200 Preps.
***Quick*-RNA™ Miniprep Plus Kit**	~10^2-10^7 cells	~100 µg	R1057 R1058	50 Preps. 200 Preps.
***Quick*-RNA™ Midiprep Kit**	~10^6-10^8 cells	~1 mg	R1056	25 Preps.
***Quick*-RNA™ 96 Kit**	~1-10^6 cells	~10 µg/well	R1052 R1053	2x 96 Preps. 4x 96 Preps.

For Individual Sale	Catalog No.	Amount
RNA Lysis Buffer	R1060-1-50 R1060-1-100	50 ml 100 ml
RNA Prep Buffer	R1060-2-10 R1060-2-25 R1060-2-100	10 ml 25 ml 100 ml
RNA Wash Buffer (concentrate)	R1003-3-6 R1003-3-12 R1003-3-24 R1003-3-48	6 ml 12 ml 24 ml 48 ml
DNase/RNase-Free Water	W1001-1 W1001-4 W1001-6 W1001-10 W1001-30	1 ml 4 ml 6 ml 10 ml 30 ml
DNase I Set (lyophilized) DNase I (250 U) & DNA Digestion Buffer (4 ml)	E1010	1 set
DNA/RNA Shield™ (2X concentrate)	R1200-25 R1200-125	25 ml 125 ml
PK Digestion Buffer	R1200-1-5 R1200-1-20	5 ml 20 ml
Proteinase K (lyophilized) (supplied with Proteinase K Storage Buffer)	D3001-2-5 D3001-2-20	5 mg set 20 mg set
Spin-Away™ Filters	C1006-50-F C1006-250-F	50 250
Zymo-Spin™ IIICG Columns	C1006-50-G C1006-250-G	50 250
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 500 1000

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