

## EXTRAClean Single Cell RNA Purification Kit

Product # 74900

Product Insert

Norgen's EXTRAClean Single Cell RNA Purification Kit provides a rapid and sensitive method for the isolation and purification of total RNA from small input amounts of cultured animal cells, sorted cells, and micro dissected samples including laser-capture microdissection (LCM). The kit can recover RNA from as little as a single cell to  $2 \times 10^5$  cells. The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). The RNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The purified RNA is of the highest integrity and can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

### Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The EXTRAClean columns undergo stringent processing and rigorous quality control measures to minimize contamination traces, ensuring optimal results for sensitive applications such as NGS. The RNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The process involves first lysing the cells with the provided Buffer RL (please see the flow chart on page 4). Ethanol is then added to the lysate, and the solution is loaded onto a Single Cell RNA Spin Column. Norgen's resin binds RNA in a manner that depends on ionic concentrations. Thus, only the RNA will bind to the column, while the contaminating proteins will be removed in the flowthrough or retained on the top of the resin. The bound RNA is then washed with the provided Wash Solution A in order to remove any remaining impurities, and the purified total RNA is eluted with the Elution Solution A. The special design of the micro spin-column allows a small elution volume of as little as  $8 \mu\text{L}$ . The purified RNA is of the highest integrity and can be used in a number of downstream applications.

### Kit Components

Component	Product # 74900 (50 preps)
Buffer RL	40 mL
Wash Solution A	38 mL
Elution Solution A	6 mL
EXTRAClean Single Cell RNA Spin Columns	50
Collection Tubes	50
Elution tubes (1.7 mL)	50 (2 x 25)
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### Advantages

- Fast and easy processing using rapid spin-column format
- Small elution volume of  $8 \mu\text{L}$
- Isolate total RNA, from large rRNA down to microRNA (miRNA) without compromising total yield
- No phenol or chloroform extractions
- Isolate high quality total RNA from a variety of sources
- RNA can be isolated and detected from as little as a single animal cell
- EXTRAClean technology yields high quality RNA optimum for NGS applications

## Kit Specifications

Kit Specifications	
Maximum Column Binding Capacity	10 µg
Maximum Column Loading Volume	650 µL
Minimum Elution Volume	8 µL
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Maximum Amount of Starting Material: Animal Cells Laser-Captured Microdissection (LCM)	Single Cell to 2 x 10 <sup>5</sup> cells Up to 2 x 10 <sup>5</sup> cells
Time to Complete 10 Purifications	20 minutes
Average Yields HeLa Cells (1 x 10 <sup>5</sup> cells) HeLa Cells (100 cells) HeLa Cells (1 cell)	~ 1.5 µg ~ 1.5 ng ≥ 1 pg

### Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. This kit is stable for 2 years after the date of shipment.

### Quality Control

In accordance with Norgen's Quality Management System, each lot of Norgen's EXTRAClean Single Cell RNA Purification Kit is tested against predetermined specifications to ensure consistent product quality.

### Product Use Limitations

Norgen's EXTRAClean Single Cell RNA Purification Kit is designed for research purposes only. They are not intended for human or diagnostic use.

### Product Warranty and Satisfaction Guarantee

NORGEN BIOTEK CORPORATION guarantees the performance of all products in the manner described in our product manual. The customer must determine the suitability of the product for its particular use.

### Safety Information

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Safety Data Sheets (SDSs). These are available as convenient PDF files online at [www.norgenbiotek.com](http://www.norgenbiotek.com).

The **Buffer RL** contains guanidine salts and should be handled with care. Guanidine salt forms highly reactive compounds when combined with bleach, thus care must be taken to properly dispose of any of these solutions.

### Customer-Supplied Reagents and Equipment

You must have the following in order to use the EXTRAClean Single Cell RNA Purification Kit:

#### For All Protocols

- Benchtop microcentrifuge
- 96 - 100% ethanol
- β-mercaptoethanol (optional)

*For Single Cell Protocol*

- 96-well collection plate for cell collection

*For Animal Cell Protocol*

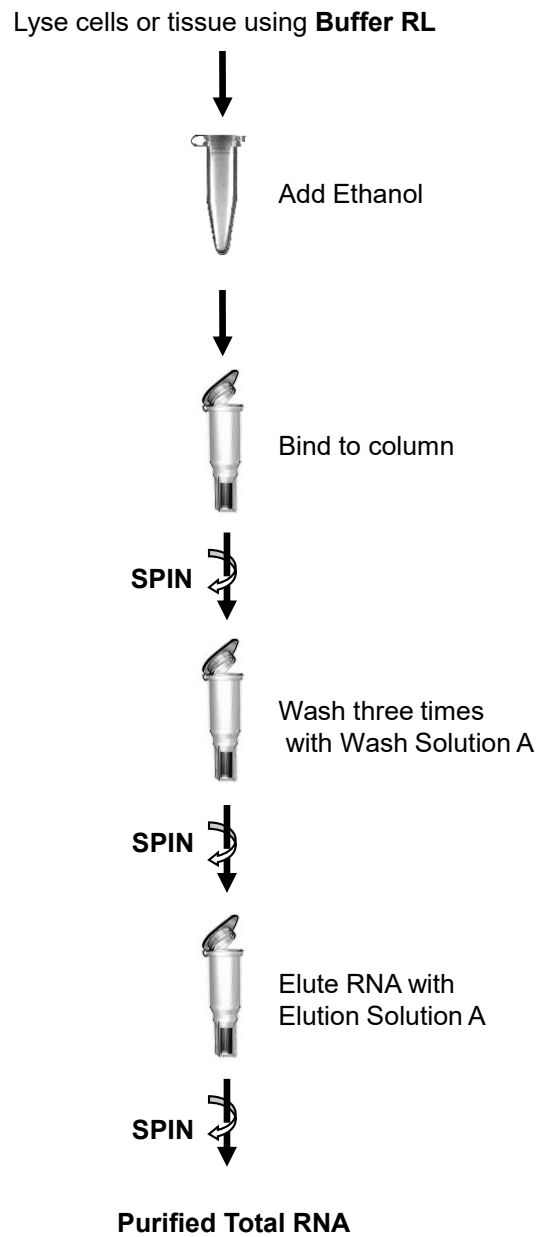
- PBS (RNase-free)
- 

*For Laser-Captured Microdissection (LCM) Protocol*

- Sterile fine forceps
- Water bath or heat block set at 42°C
- 70% ethanol

**Flowchart**

Procedure for Purifying Total RNA using Norgen's Single EXTRAClean Single Cell RNA Purification Kit



## Procedure

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5}) (r)}}$$

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.

### Working with RNA

RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware are not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.

- The RNA area should be located away from microbiological workstations
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination.
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water
- Clean all surfaces with commercially available RNase decontamination solutions
- When working with purified RNA samples, ensure that they remain on ice during downstream applications

## Section 1. Preparation of Lysate from Various Cell Types

### Notes Prior to Use

- The steps for preparing the lysate are different depending on the starting material (**Step 1**). However, the subsequent steps are the same in all cases (**Steps 2 – 6**).
- Please ensure that the correct procedure for preparing the lysate from your starting material is followed.
- All centrifugation steps are carried out in a benchtop microcentrifuge at 20,800 x g (~14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.
- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution A** by adding 90 mL of 96 - 100% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution A**. This will give a final volume of 128 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- **Optional:** The use of  $\beta$ -mercaptoethanol in lysis is highly recommended for most animal tissues (LCM samples), particularly those known to have a high RNase content (ex: pancreas). It is also recommended for users who wish to isolate RNA for sensitive downstream applications. Add 10  $\mu$ L of  $\beta$ -mercaptoethanol (provided by the user) to each 1 mL of Buffer RL required.  $\beta$ -mercaptoethanol is toxic and should be dispensed in a fume hood. Alternatively, the Buffer RL can be used as provided.
- It is important to work quickly during this procedure.

## 1A. Lysate Preparation from Single Animal Cell

### Notes Prior to Use

- Freshly sorted cells are recommended for this protocol.
- Cells can be sorted by various procedures including classic flow cytometry and fluorescence-activated cell sorting (FACS)
- Ensure that all the components of the flow cytometer (such as dip tube, septa, flow cell, all tubing lines, and nozzles) are decontaminated for RNases using an appropriate reagent

### 1A. Cell Lysate Preparation from Single Cell or Sorted Cells

- a. Aliquot 100  $\mu\text{L}$  of **Buffer RL** to an RNase-free microcentrifuge tube or into each well of a 96-well collection plate.
- b. Sort the cells directly into the aliquoted **Buffer RL**. Mix by pipetting up and down a few times. If the cells are collected onto a 96-well plate, transfer the lysate to an RNase-free microcentrifuge tube.
- c. Add 100  $\mu\text{L}$  of 70% ethanol (provided by the user) to the lysate. Mix by vortexing.

## 1B. Lysate Preparation from Cultured Animal Cells

### Notes Prior to Use

- The maximum recommended input of cells is  $2 \times 10^5$ . A hemocytometer can be used in conjunction with a microscope to count the number of cells. As a general guideline, each well of a confluent 96-well plate of HeLa cells will contain  $2\text{-}5 \times 10^4$  cells.
- Cell pellets can be stored at  $-70^\circ\text{C}$  for later use or used directly in the procedure. Determine the number of cells present before freezing.
- Frozen pellets should be stored for no longer than 2 weeks to ensure that the integrity of the RNA is not compromised.
- Frozen cell pellets should not be thawed prior to beginning the protocol. Add the Buffer RL directly to the frozen cell pellet (**Step 1B(ii) c**).

### 1B(i). Cell Lysate Preparation from Cells Growing in a Monolayer

- a. Aspirate media and wash cell monolayer with an appropriate amount of PBS. Aspirate PBS.
- b. Add 200  $\mu\text{L}$  of **Buffer RL** directly to culture plate.
- c. Lyse cells by gently tapping culture dish and swirling buffer around plate surface for five minutes.
- d. Transfer lysate to a microcentrifuge tube.
- e. Add 120  $\mu\text{L}$  of 96 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **Proceed to Step 2.**

### 1B (ii). Cell Lysate Preparation from Cells Growing in Suspension and Lifted Cells

- a. Transfer cell suspension to an RNase-free tube (not provided) and centrifuge at no more than  $425 \times g$  ( $\sim 2,000$  RPM) for 10 minutes to pellet cells.
- b. Carefully decant the supernatant.  
**Note:** For inputs of over  $10^5$  cells, 5-10  $\mu\text{L}$  of media may be left behind with the pellet in order to ensure that the pellet is not dislodged. For inputs of fewer than  $10^5$  cells, 30-50  $\mu\text{L}$  of media may be left behind in order to ensure that the pellet, which could be invisible, is not dislodged.
- c. Add 350  $\mu\text{L}$  of **Buffer RL** to the pellet. Lyse cells by vortexing for 15 seconds. Ensure that the entire pellet is completely dissolved before proceeding to the next step.
- d. Add 200  $\mu\text{L}$  of 96 - 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds. **Proceed to Step 2.**
- e. Spin down the lysate to ensure that there is nothing sticking on the caps. Proceed to Section 2.

## 1C. Lysate Preparation from Laser-Captured Microdissection (LCM)

### Notes Prior to Use

- LCM samples obtained from frozen sections are recommended. Formalin-Fixed, Paraffin-Embedded sections may also be used. However, RNA isolated from FFPE samples generally has poorer quality than that from frozen sections.

### 1C. Cell Lysate Preparation from Laser-Captured Microdissection (LCM)

- Aliquot 300  $\mu$ L of **Buffer RL** to an RNase-free microcentrifuge tube.
- Remove the thermoplastic film containing the captured cells using sterile fine forceps. Carefully submerge the sample into the aliquoted **Buffer RL**. Close the microcentrifuge cap.
- Incubate the sample at 42°C for 30 minutes. Apply vortex for 15 seconds after every 10 minutes.
- At the end of the incubation, vortex the tube one more time for 15 seconds. The thermoplastic film may be removed at this point using sterile fine forceps. Otherwise, proceed to **Step 1Ce**.
- Add 300  $\mu$ L of 70% ethanol (provided by the user) to the lysate. Vortex to mix. **Proceed to Step 2.**

## Section 2. Total RNA Purification from All Types of Lysates

**Note:** The remaining steps of the procedure for the purification of total RNA are the same from this point forward for all the different types of lysates.

### 2. Binding RNA to Column

- Assemble an EXTRAClean Single Cell RNA Spin Column with one of the provided collection tubes.
- Apply up to 600  $\mu$ L of the lysate with the ethanol (from **Step 1**) onto the EXTRAClean Single Cell RNA Spin column and centrifuge for 1 minute at **3,500 x g (~6,000 RPM)**.

**Note:** Ensure the entire lysate volume has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute at **20,800 x g (~14,000 RPM)**.

- Discard the flowthrough. Reassemble the EXTRAClean Single Cell RNA Spin column with its collection tube.
- Depending on your lysate volume, repeat **Step 2b** and **2c** as necessary.

#### Optional Step:

Norgen's EXTRAClean Single Cell RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional **On-Column DNA Removal Protocol** is provided in **Appendix A** for maximum removal of residual DNA that may affect sensitive downstream applications. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step. This step should be performed at this point in the protocol.

### 3. Column Wash

- Apply 400  $\mu$ L of **Wash Solution A** to the EXTRAClean Single Cell RNA Spin column and centrifuge for 1 minute at **20,800 x g (~14,000 RPM)**.  
**Note:** Ensure the entire Wash Solution A has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.
- Discard the flowthrough and reassemble the EXTRAClean Single Cell RNA Spin column with its collection tube.
- Repeat steps **3a** and **3b** to wash column a second time.

- d. Wash EXTRAClean Single Cell RNA Spin column a third time by adding another 400  $\mu\text{L}$  of **Wash Solution A** and centrifuging for 1 minute at **20,800 x g (~14,000 RPM)**.
- e. Discard the flowthrough and reassemble the EXTRAClean Single Cell RNA Spin column with its collection tube.
- f. Spin the EXTRAClean Single Cell RNA Spin column for 2 minutes at **20,800 x g (~14,000 RPM)** in order to thoroughly dry the resin. Discard the collection tube.

#### 4. RNA Elution

- a. Place the EXTRAClean Single Cell RNA Spin column into a fresh 1.7 mL Elution tube provided with the kit.
- b. Add 8 - 20  $\mu\text{L}$  of **Elution Solution A** to the column.

**Note:** For higher concentrations of RNA, a lower elution volume may be used. A minimum volume of 8  $\mu\text{L}$  is recommended

- c. Centrifuge for 1 minute at **425 x g (~2,000 RPM)**, followed by 1 minute at **20,800 x g (~14,000 RPM)**. Note the volume eluted from the column. If the entire volume has not been eluted, spin the EXTRAClean Single Cell RNA Spin column at 20,800 x g (~14,000 RPM) for 1 additional minute.

#### 5. Storage of RNA

The purified RNA sample may be stored at  $-20^{\circ}\text{C}$  for a few days. It is recommended that samples be placed at  $-70^{\circ}\text{C}$  for long term storage.

#### 6. Quantification of RNA

For cell inputs of  $\geq 1 \times 10^5$  cells, it is possible to quantify RNA using a nanospectrophotometer (such as Thermo Scientific's NanoDrop). For cell inputs between  $10^2$  and  $10^5$  cells, RNA quantification requires a highly sensitive fluorescence-based system (such as Life Technologies' RiboGreen® RNA Assay kit). For cell inputs between a single cell and  $10^4$  cells, quantification could be performed using RT-qPCR of an RNA transcript of high abundance (such as GAPDH or 5S rRNA) with a standard curve generated with total RNA of known concentration.

## Appendix A

### Protocol for Optional On-Column DNA Removal

Norgen's EXTRAClean Single Cell RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional protocol is provided below for maximum removal of residual DNA that may affect sensitive downstream applications. It is recommended that Norgen's RNase-Free DNase I Kit (Product # 25710) be used for this step.

1. For every on-column reaction to be performed, prepare a mix of 15  $\mu\text{L}$  of **DNase I** and 100  $\mu\text{L}$  of **Enzyme Incubation Buffer** using Norgen's RNase-Free DNase I Kit (Product # 25710). Mix gently by inverting the tube a few times. **DO NOT VORTEX**.

**Note:** If using an alternative DNase I, prepare a working stock of 0.25 Kunitz unit/ $\mu\text{L}$  RNase-free DNase I solution according to the manufacturer's instructions. A 100  $\mu\text{L}$  aliquot is required for each column to be treated.

2. Perform the appropriate Total RNA Isolation Procedure for your starting material up to and including "**Binding to Column**" (Steps 1 and 2 of all protocols)
3. Apply 400  $\mu\text{L}$  of **Wash Solution A** to the EXTRAClean micro spin column and centrifuge for 2 minutes. Discard the flowthrough. Reassemble the spin column with its collection tube.

4. Apply 100  $\mu$ L of the RNase-free DNase I solution prepared in Step 1 to the column and centrifuge at 20,800 x g (~14 000 RPM) for 1 minute.
 

**Note:** Ensure that the entire DNase I solution passes through the column. If needed, spin at 20,800 x g (~14 000 RPM) for an additional minute.
5. After the centrifugation in Step 4, pipette the flowthrough that is present in the collection tube back onto the top of the column.
 

**Note:** Ensure Step 5 is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species.
6. Incubate the column assembly at 25 - 30°C for 15 minutes.
7. Without any further centrifugation, proceed directly to the second wash step in the “**Column Wash**” section (Step 3c).

### Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Incomplete lysis of cells or tissue	Ensure that the appropriate amount of Buffer RL was used for the amount of cells or tissue.
	Column has become clogged	Do not exceed the recommended amounts of starting materials. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also “Clogged Column” below.
	An alternative elution solution was used	It is recommended that the Elution Solution A supplied with this kit be used for maximum RNA recovery.
	Ethanol was not added to the lysate	Ensure that the appropriate amount of ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution A	Ensure that 90 mL of 96-100% ethanol is added to the supplied Wash Solution A prior to use.
	Low RNA content in cells or tissues used	Different tissues and cells have different RNA contents, and thus the expected yield of RNA will vary greatly from these different sources. Please check literature to determine the expected RNA content of your starting material.
	Cell Culture: Cell monolayer was not washed with PBS	Ensure that the cell monolayer is washed with the appropriate amount of PBS in order to remove residual media from cells.
	LCM: Sample was not incubated at 42°C for 30 minutes	Ensure that the incubation at 42°C for the removal and lysis of cells from the thermoplastic film.

<b>Problem</b>	<b>Possible Cause</b>	<b>Solution and Explanation</b>
Clogged Column	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 15°C may cause precipitates to form that can cause the columns to clog.
RNA does not perform well in downstream applications	RNA was not washed 3 times with the provided Wash Solution A	Traces of salt from the binding step may remain in the sample if the column is not washed 3 times with Wash Solution A. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
RNA is Degraded	RNase contamination	RNases may be introduced during the use of the kit. Ensure proper procedures are followed when working with RNA. Please refer to “ <i>Working with RNA</i> ” at the beginning of this user guide.
	Procedure not performed quickly enough	In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly. This is especially important for the Cell Lysate Preparation Step in the Animal Tissue protocol, since the RNA in animal tissues is not protected after harvesting until it is disrupted and homogenized.
	Improper storage of the purified RNA	For short term storage RNA samples may be stored at –20°C for a few days. It is recommended that samples be stored at –70°C for longer term storage.
	Frozen tissues or cell pellets were allowed to thaw prior to RNA isolation	Do not allow frozen tissues to thaw prior to grinding with the mortar and pestle in order to ensure that the integrity of the RNA is not compromised.
	Starting material may have a high RNase content	For starting materials with high RNAase content, it is recommended that β-mercaptoethanol be added to the Buffer RL.
Genomic DNA contamination	Large amounts of starting material used	Perform RNase-free DNaseI digestion on the RNA sample after elution to remove genomic DNA contamination. It is recommended that Norgen’s RNase-Free DNase I Kit (Product # 25710) be used for this step.

Related Products	Product #
Proteinase K – 2 Vials	17904
RNase-Free DNase I Kit	25710
Total RNA Purification Kit	17200
Total RNA Purification 96-Well Kit	24300
Total RNA Purification Maxi Kit	26800
Animal Tissue RNA Purification Kit	25700
Plant/Fungi Total RNA Purification Kit	25800
RNA/Protein Purification Kit	24100
RNA/DNA/Protein Purification Kit	24000
Cytoplasmic & Nuclear RNA Purification Kit	21000
Leukocyte RNA Purification Kit	21200
microRNA Purification Kit	21300
100b RNA Ladder	15002
1kb RNA Ladder	15003

### Technical Support

Contact our Technical Support Team between the hours of 9:00 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website ([www.norgenbiotek.com](http://www.norgenbiotek.com)) or through email at [techsupport@norgenbiotek.com](mailto:techsupport@norgenbiotek.com).

Norgen's purification technology is patented and/or patent pending. See [www.norgenbiotek.com/patents](http://www.norgenbiotek.com/patents)

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