



UltraClean™ Microbial RNA Isolation Kit

Catalog

Catalog No.	Quantity
15800-50	50 preps
15800-250	250 preps

Instruction Manual

Introduction

The UltraClean™ Microbial RNA Isolation Kit is designed to isolate high-quality RNA from microorganisms. A variety of microorganisms including bacterial spores and fungal types have been tested with this kit.

Microbial cells are added to a bead beating tube containing beads, bead solution and lysis solution. The cells are lysed by mechanical action on a vortex. The lysate is centrifuged through a spin filter. RNA is bound to the spin filter due to the high salt nature of the binding buffer. The filter is washed and the RNA is recovered in certified RNase-free elution buffer.

This kit is for research purposes only. Not for diagnostic use.

Version: 03172008

Technical information: Toll free 1-800-606-6246, or 1-760-929-9911 email: technical@mobio.com



Equipment required:

Microcentrifuge (10,000 x g)
 65°C water bath
 Pipettor (volumes required 50 µl–200 µl, 100 µl-1000 µl)
 Vortex

Kit Contents

Component	Kit Catalog # 15800-50		Kit Catalog # 15800-250	
	Catalog #	Amount	Catalog #	Amount
MicroRNA Bead Tubes (contain 0.25g of beads)	15800-50-BT	50	15800-250-BT	250
Solution MR1	15800-50-1	16 ml	15800-250-1	80 ml
Solution MR2	15800-50-2	850 µl	15800-250-2	4 ml
Solution MR3	15800-50-3	28 ml	15800-250-3	130 ml
Solution MR4	15800-50-4	15 ml	15800-250-4	65 ml
Solution MR5	15800-50-5	32 ml	15800-250-5	3 x 33 ml
RNase-free Water	15800-50-6	5 ml	15800-250-6	15 ml
Spin Filters (units in 2 ml tubes)	15800-50-SF	50	15800-250-SF	250
2 ml Collection Tubes	15800-50-T	200	15800-250-T	1000

Kit Storage

Store DTT (Solution MR2) at 4°C and all other reagents at room temperature.

Precautions

Please wear gloves when using this product. Avoid all skin contact with reagents in this kit. In case of contact, wash thoroughly with water. Do not ingest. See Material Safety Data Sheets for emergency procedures in case of accidental ingestion or contact. All MSDS information is available upon request (760-929-9911) or on our web site at www.mobio.com. Reagents labeled flammable should be kept away from open flames and sparks.

Make sure the 2 ml MicroRNA screw cap tubes rotate freely in your centrifuge without rubbing. Do not spin the Bead Tubes in excess of 10,000 x g.

WARNINGS:

Solution MR5 contains ethanol and it is flammable.

Solution MR2 contains DTT a strong reducing reagent. Avoid bringing strong oxidizers, like bleach, in contact with this solution.

Solution MR1 contains Guanidine thiocyanate. Avoid skin contact.



Protocol

Please wear gloves at all times

Preparation:

Before starting, check **Solution MR2**. If precipitated, heat at 65°C for approximately 5 minutes with periodic mixing until completely dissolved.

1. Add 1.8 ml of microbial culture to a **2 ml Collection Tube** (provided) and centrifuge at 10,000 x *g* for 30 seconds. Decant the supernatant and spin the tubes one more time for 30 seconds and remove the media supernatant completely with a pipet tip.
2. Resuspend the cell pellet in 300 µl of **Solution MR1** and gently vortex to mix. Transfer resuspended cells to a **MicroRNA Bead Tube**.
3. Add 15 µl of **Solution MR2** to the **MicroRNA Bead Tube** and vortex briefly to mix.
4. Heat the tubes at 65°C for 10 minutes
5. Secure bead tubes horizontally using the MO BIO Vortex Adapter tube holder for the vortex (MO BIO catalog# 13000-V1) or secure tubes horizontally on a flat-bed vortex pad with tape.
6. Vortex at maximum speed for 10 minutes.
7. Make sure the 2 ml **MicroRNA Bead Tubes** rotate freely in your centrifuge without rubbing. Centrifuge these tubes at 10,000 x *g* for 30 seconds. **CAUTION:** Be sure not to exceed 10,000 x *g* or tubes may break.
8. Transfer the supernatant to a clean **2 ml Collection Tube** (provided). Carry over of residual beads is normal. **Note:** Expect 300 to 350 µl of supernatant.
9. Add 500 µl of **Solution MR3** (Lysis buffer) to the supernatant. Vortex 5 seconds. Then add 250µl of **Solution MR4** (Lysis buffer). Incubate at 4°C for 5 minutes.
10. Centrifuge the tubes for 1 minute at 10,000 x *g*.
11. Avoiding the pellet, transfer the entire volume of supernatant to a clean **2 ml Collection Tube** (provided). You can expect approximately 1000 µl in volume.
12. Load about 650 µl into **Spin Filter** and centrifuge at 10,000 x *g* for 30 seconds. Discard the flow through and add the remaining supernatant to the **Spin Filter** and centrifuge at 10,000 x *g* for 30 seconds. **NOTE:** A total of 2 loads for each sample processed are required. Discard all flow through liquid.
13. Add 300 µl of **Solution MR5** and centrifuge for 30 seconds at 10,000 x *g*.
14. Discard the flow through.
15. Centrifuge again for 1 minute at 10,000 x *g*.
16. Being careful not to splash liquid on the filter basket, place spin filter basket in a new **2 ml Collection Tube** (provided).
17. Add 50 µl of **RNase-free Water** to the center of the white filter membrane.
18. Centrifuge 30 seconds.
19. Discard **Spin Filter**. RNA in the tube may contain some amount of DNA. Digestion with RNase free DNase may be necessary.

We recommend storing RNA frozen (-20°C). **Solution M5** contains no EDTA.

Thank you for choosing the UltraClean™ Microbial RNA Isolation Kit



Hints and Troubleshooting Guide

Concentrating the RNA

The final volume will be 50 μ l. If this is too dilute for your purposes, add 5 μ l of 5M NaCl and mix. Then add 100 μ l of 100% cold ethanol. Mix. Centrifuge at 10,000 x g for 5 minutes. Decant all liquid. Dry residual ethanol in a speed vac or dessicator or ambient air. Resuspend precipitated RNA in desired volume.

RNA floats out of well when loaded on a gel

You may have inadvertently transferred some residual Solution MR5 into the final sample. Prevent this by being careful in step 16 not to transfer liquid onto the bottom of the spin filter basket. Ethanol precipitation is the best way to remove residues of Solution MR5. (See concentrating RNA above)

Storing RNA

RNA is eluted in water therefore it must be stored at -20°C or it may degrade. RNA can be eluted in TE but the EDTA may inhibit reactions such as RT-PCR.



Other UltraClean™ Kits available from MO BIO Laboratories, Inc.

<u>Kit description</u>	<u>Cat. number</u>
Plasmid Prep Kits	
6 minute Mini Plasmid Prep Kit (100 preps)	12300-100
6 minute Mini Plasmid Prep Kit (250 preps)	12300-250
25-50 ml Plasmid Prep Kit (20 preps)	12700-20
25-50 ml Plasmid Prep Kit (50 preps)	12700-50
250-500 ml Plasmid Prep Kit (10 preps)	12600-10
250-500 ml Plasmid Prep Kit (20 preps)	12600-20
Endotoxin-Free Plasmid Prep Kits	
Endotoxin-free Mini Prep Kit (100 preps)	12311-100
Endotoxin-free Mini Prep Kit (250 preps)	12311-250
Endotoxin-free Midi Prep Kit (10 preps)	12711-10
Endotoxin-free Maxi Prep Kit (10 preps)	12611-10
DNA Purification Kits	
Agarose Gel DNA Purification Kit (300 preps)	12100-300
Agarose Gel-Spin DNA Purification (100 preps)	12400-100
Agarose Gel-Spin DNA Purification (250 preps)	12400-250
PCR Clean-Up Kit (100 preps)	12500-100
PCR Clean-Up Kit (250 preps)	12500-250
DNA Isolation Kits	
DNA Blood Isolation Kit (100 preps)	12000-100
DNA BloodSpin Kit (50 preps)	12200-50
DNA BloodSpin Kit (250 preps)	12200-250
Mega BloodSpin Kit (10 preps)	12210-10
Soil DNA Isolation Kit (50 preps)	12800-50
Soil DNA Isolation Kit (100 preps)	12800-100
Soil DNA Mega Prep Kit (10 preps)	12900-10
Fecal DNA Isolation Kit (50 preps)	12811-50
Fecal DNA Isolation Kit (100 preps)	12811-100
Microbial DNA Isolation Kit (50 preps)	12224-50
Microbial DNA Isolation Kit (250 preps)	12224-250
Plant DNA Isolation Kit (50 preps)	13000-50
Plant DNA Isolation Kit (250 preps)	13000-250
Tissue DNA Isolation Kit (50 preps)	12334-50
Tissue DNA Isolation Kit (250 preps)	12334-250
Water DNA Isolation Kit (10 preps)	14800-10
Water DNA Isolation Kit (25 preps)	14800-25
Forensic DNA Kit- Single prep format (10 preps)	14000-10
Forensic DNA Kit- Single prep format (20 preps)	14000-20
RNA Isolation Kits	
Tissue RNA Isolation Kit (50 preps)	15000-50
Tissue RNA Isolation Kit (250 preps)	15000-250
Plant RNA Isolation Kit (20 preps)	13300-20
Plant RNA Isolation Kit (50 preps)	13300-50
Microbial RNA Isolation Kit (50 preps)	15800-50
Microbial RNA Isolation Kit (250 preps)	15800-250
Growth Media	
TB DRY (1 kg) Terrific Broth powder	12105-1
LB (1 kg) LB powder (Miller)	12106-1
LB Agar (1 kg) LB Agar Powder (Miller)	12107-1

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Contact Information -

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Ordering Information:

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For the distributor nearest you, go to our web site at www.mobio.com/distributors/