



RNA PowerSoil™ Total RNA Isolation Kit*

Catalog No.	Quantity
12866-25	25 Preps

Instruction Manual

Introduction

MO BIO Laboratories' RNA PowerSoil™ Total RNA Isolation Kit is designed to isolate total RNA from organisms found in soil. The patent pending properties of the kit permit consistent removal of humic substances, fulvic acids, and other RT-PCR inhibitors from soil purified RNA. Diverse soil types, including compost, manure, estuary sediment, and other soil types high in organic content, have successfully provided biologically intact and RT-PCR amplifiable RNA using this kit. The RNA PowerSoil™ Total RNA Isolation Kit reliably provides RNA for experiments requiring qualitative and quantitative RT-PCR analysis.

The dynamics of microbial diversity in soils will vary with the microbial populations and their metabolic status. This in turn is a function of soil composition, soil moisture content, the amount of sunlight, the availability of nutrients and other environmental factors. The RNA PowerSoil™ Total RNA Isolation Kit is designed to extract RNA from the total soil microflora and microfauna, including metabolically active, metabolically dormant and dead organisms. As such, the RNA PowerSoil™ Total RNA Isolation Kit will provide the best possible representation of the population of soil organisms and RNA composition.

IMPORTANT: This kit requires user provided phenol:chloroform:isoamyl alcohol (25:24:1, pH 6.5 – 8.0). This reagent can be purchased from Amresco, Incorporated or VWR International (see List of Recommended Vendors for Phenol:Chloroform:Isoamyl Alcohol in the Additional Information Section). The phenol:chloroform:isoamyl alcohol may also be user made (please refer to, Preparing Phenol:Chloroform:Isoamyl Alcohol in the Additional Information Section for preparation instructions). NOTE: Phenol and phenol:chloroform:isoamyl alcohol are subject to oxidation reactions that cause them to become yellow or pink colored, which serves as an indicator that the phenol is NOT useable for RNA extraction. Using colored phenol or colored phenol:chloroform:isoamyl alcohol will result in quality compromised RNA. Prior to each use, a sample of the phenol:chloroform:isoamyl alcohol should be placed in a clear container and its clarity determined. When not in use, store the phenol:chloroform:isoamyl alcohol at 4°C in the dark. Securely cap when not in use and do not expose to light for prolonged periods.

*PATENT PENDING

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

Version: 10202006

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



Required Equipment:

- Centrifuge capable of centrifuging 15ml tubes (2500 x g minimum)
- Microcentrifuge (13,000 x g)
- Pipettor (20 µl to 1000 µl)
- Serological pipettes (1 ml and 10 ml)
- Heat block (set at 45°C) (optional)
- Vortex
- RNase-Free gloves (MO BIO Laboratories catalog number 1555-S (small), 1555-M (medium) and 1555-L (large))
- Lab Cleaner for RNase Removal (MO BIO Laboratories catalog number 12095-250, 12095-500)
- Vortex Adapter (MO BIO Laboratories catalog number 13000-V1-15 for Vortex Genie 2 or 13000-LV2-15 for Labnet Vortex)

Kit Contents

Component	Kit Catalog# 12866-25	
	Catalog #	Amount
RNA PowerSoil™ Bead Tubes (with 1.5 g beads)	12866-25-PBT	25
RNA PowerSoil™ Bead Solution	12866-25-BS	69 ml
RNA PowerSoil™ Solution SR1	12866-25-1	7 ml
RNA PowerSoil™ Solution SR2	12866-25-2	22 ml
RNA PowerSoil™ Solution SR3	12866-25-3	42 ml
RNA PowerSoil™ Solution SR4	12866-25-4	165 ml
RNA PowerSoil™ Solution SR5	12866-25-5	110 ml
RNA PowerSoil™ Solution SR6	12866-25-6	28 ml
RNA PowerSoil™ Solution SR7	12866-25-7	3 ml
RNA PowerSoil™ RNA Capture Columns	12866-25-SF	25
RNA PowerSoil™ 15 ml Collection Tubes	12866-25-T1	100
RNA PowerSoil™ 2.2 ml Collection Tubes	12866-25-T2	25

Kit Storage

Kit components are stored at room temperature (15 to 30°C).

Precautions

Wear gloves, laboratory coat and safety glasses when using this product. Avoid skin contact with kit reagents. In case of contact, wash the affected area thoroughly with soap and water. Do not ingest. See Material Safety Data Sheets (MSDS) for emergency procedures in case of accidental contact or ingestion. MSDS information is available upon request (760-929-9911) or at www.mobio.com. Reagent SR4 is flammable and should be kept away from open flames and sparks.

WARNING: Phenol: chloroform: isoamyl alcohol [User supplied] is a caustic solution. User should review the Material Safety Data Sheets and accident procedures provided by the phenol vendor for this reagent prior to handling. To avoid injury, wear gloves, goggles and a lab coat. Use in a fume hood and do not inhale vapors. Follow local ordinances for disposal of phenol waste. Chloroform is a carcinogen. In the event of an accident, seek medical attention immediately.

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RNA PowerSoil™ Total RNA Isolation Kit Protocol

Wear RNase-Free gloves at all times and remove RNase from the work area using Lab Cleaner for RNase Removal.

1. Add up to 2 g of soil to the 15 ml Bead Tube (provided). **Note:** Please refer to Additional Information Section for information regarding the amount of soil to process.
2. Add 2.5 ml of Bead Solution to the Bead Tube and vortex to mix.
3. Add 0.25 ml of Solution SR1 to the Bead Tube and vortex to mix.
4. Add 0.8 ml of Solution SR2 and place the Bead Tube on the Vortex Adapter (MO BIO Laboratories Catalog # 13000-V1-15 for Vortex Genie 2 or 13000-LV2-15 for Labnet Vortex) and vortex at maximum speed for 5 minutes.
5. Remove the Bead Tube from the Vortex Adapter and add 3.5 ml of phenol:chloroform:isoamyl alcohol (pH 6.5 – 8.0, [User supplied]) and vortex to mix until the biphasic layer disappears.
6. Place the Bead Tube on the Vortex Adapter and vortex at maximum speed for 10 minutes.
7. Remove the Bead Tube from the Vortex Adapter and centrifuge at 2500 x g for 10 minutes at room temperature.
8. Remove the Bead Tube from the centrifuge and carefully transfer the upper aqueous phase (avoiding the interphase and lower phenol layer) to a clean 15 ml Collection Tube (provided). The thickness of the interphase will vary depending on the type of soil used. Discard the phenol:chloroform:isoamyl alcohol in an approved waste receptacle. NOTE: The biphasic layer will be thick and firm in soils high in organic matter and may need to be pierced to remove the bottom phenol layer.
9. Add 1.5 ml of Solution SR3 to the aqueous phase and vortex to mix. Incubate at 4°C for 10 minutes.
10. Centrifuge at 2500 x g for 10 minutes at room temperature.
11. Transfer the supernatant, without disturbing the pellet, to a new 15 ml Collection Tube (provided).
12. Add 5 ml of Solution SR4 to the Collection Tube containing the supernatant, invert or vortex to mix, and incubate at -20°C for 30 minutes.
13. Centrifuge at 2500 x g for 30 minutes at room temperature.
14. Decant the supernatant and invert the 15 ml Collection Tube on a paper towel for 5 minutes. NOTE: Depending on soil type, the pellet may be large and/or dark in color.
15. Add 1 ml of Solution SR5 to the 15 ml Collection Tube and resuspend the pellet completely. (NOTE: Depending on the soil type, the pellet may be difficult to resuspend. Resuspension may be aided by placing the tubes in a heat block or water bath at 45°C for 10 minutes, followed by vortexing. Repeat until the pellet is resuspended.)
16. Prepare one RNA Capture Column (provided) for each RNA Isolation Sample:
 - a. Remove the cap of a 15 ml Collection Tube (provided) and place the RNA Capture Column inside the 15 ml Collection Tube. The column will hang in the 15ml tube.
 - b. Add 2 ml of Solution SR5 to the RNA Capture Column and allow it to gravity flow through the column and collect in the 15 ml Collection Tube. Allow Solution SR5 to completely flow through the column (OPTIONAL: The Collection Tube may be emptied after Solution SR5 has completely flowed through the column. NOTE: DO

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NOT ALLOW THE COLUMN TO DRY OUT PRIOR TO LOADING THE RNA ISOLATION SAMPLE.)

17. Add the RNA Isolation Sample from Step 15 onto the RNA Capture Column and allow it to gravity flow through the column. Collect the flow through in the 15 ml Collection Tube.
18. Wash the column with 1 ml of Solution SR5. Allow it to gravity flow and collect the flow through in the 15 ml Collection Tube.
19. Transfer the RNA Capture Column to a new 15 ml Collection Tube (provided) and add 1 ml of Solution SR6 to the RNA Capture Column to elute the bound RNA into the 15 ml Collection Tube. Allow Solution SR6 to gravity flow into the 15 ml Collection Tube.
20. Transfer the eluted RNA to a 2.2 ml Collection Tube (provided) and add 1 ml of Solution SR4. Invert at least once to mix and incubate at -20°C for 10 minutes.
21. Centrifuge the 2.2 ml Collection Tube at 13,000 x g for 15 minutes at room temperature to pellet the RNA.
22. Decant the supernatant and invert the 2.2 ml Collection Tube onto a paper towel for 10 minutes to air dry the pellet.
23. Resuspend the RNA pellet in 100 µl of Solution SR7. (NOTE: Although DNA carryover does not occur with the majority of soil types, certain soils high in organic matter may present unique carryover situations. In situations where the absence of DNA contamination is critical, the purified RNA should be tested for potential DNA carryover by performing PCR with qualified primers on the isolated RNA without performing prior reverse transcription amplification. The absence of a detectable amplification fragment by agarose electrophoresis indicates the absence of detectable carryover DNA. In the event DNA is detected, DNase treatment of the isolated RNA is recommended; see Additional Information Section for instruction).

Thank you for choosing the RNA PowerSoil™ Total RNA Isolation Kit.

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Suggested Protocol for Formaldehyde Agarose Gel Electrophoresis

Solutions Needed:

10x Formaldehyde Agarose Gel Buffer

200mM 3-[N-morpholino] propanesulfonic acid (MOPS, free acid)
50 mM Sodium Acetate
10 mM EDTA
pH to 7.0 with Sodium Hydroxide
Prevent exposure to light (store in a dark bottle).

1x Formaldehyde Agarose Gel Buffer (1L)

100 ml 10x Formaldehyde Agarose Gel Buffer
20 ml 37% (12.3M) Formaldehyde
880 ml DEPC treated water

5x RNA Loading Dye

16 µl of Saturated Aqueous Bromophenol Blue Solution
80 µl of 5M EDTA, pH 8.0
720 µl of 37% (12.3M) Formaldehyde
2 ml of 100% Glycerol
3084 µl of Formamide
4 ml of 10x Formaldehyde Agarose Gel Buffer

Formaldehyde Agarose Gel Preparation

To make a 1.2% Formaldehyde Agarose Gel with 100 ml volume, mix the following:

1.2 g of Agarose
10 ml of 10x Formaldehyde Agarose Gel buffer
90 ml of DEPC treated water

Heat the mixture in a microwave oven to melt the agarose. Cool to 65°C in a water bath. Add 1.8 ml of 37% (12.3 M) formaldehyde and 2 µl of 5 mg/ml ethidium bromide solution. Swirl to mix and pour into a gel box (NOTE: Do not breath formaldehyde fumes. Pour the melted agarose in a fume hood or cover the gel tray with plastic wrap immediately after pouring.). Pre-run the gel for 30 minutes at 5 – 7 V/cm in 1x Formaldehyde Agarose Gel Buffer before loading the samples. After loading, run the gel at 5 – 7 V/cm until the bromophenol blue is approximately two-thirds of the distance to the gel edge (See RNA Sample Preparation).

RNA Sample Preparation

RNA analyzed by TAE or TBE native gel electrophoresis may be loaded and analyzed using native gel electrophoresis loading and gel running buffers.

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RNA analyzed by formaldehyde agarose gel electrophoresis must be denatured before application to the gel. Add 1 volume of 5x RNA loading dye for each 4 volumes of RNA sample (i.e. 2 μ l of 5x RNA loading dye for each 8 μ l of RNA sample). Mix the sample and loading dye, and centrifuge to collect the sample at the bottom of the tube. Incubate the sample at 65°C for 3 – 5 minutes, chill on ice and centrifuge to collect the sample at the tube bottom. Load the sample into the Formaldehyde Agarose Gel and run the gel at 5-7 V/cm in 1x Formaldehyde Agarose Gel Buffer for approximately 90 minutes.

References

1. Beintema, J.J., Campagne, R.N., and Gruber, M. 1973. *Biochim. Biophys. Acta* 310: 148-160
Kaplan, B.B., Bernstein, S.L., and Gioio, A.E. 1979. *Biochem. J.* 183-181-184.



Additional Information

Soil Types and Soil Amount Processed

The yield and purity of RNA will depend on the soil type processed. The RNA PowerSoil™ Total RNA Isolation Kit has been validated with diverse soil types that represent a wide range of physical, chemical and biological characteristics. In our experience, it is possible to use up to a maximum of 2 g for most soil types. For soils with high organic content, 1 g of soil typically gives an adequate amount of RNA while reducing the potential for DNA carryover during purification.

Phase Separation of Phenol:Chloroform:Isoamyl Alcohol

To ensure effective phenol:chloroform:isoamyl alcohol and aqueous phase separation, centrifuge 15 ml tubes at 2,500 x g at room temperature for at least 10 minutes (Step 7). Following centrifugation, the interphase thickness between the phenol:chloroform:isoamyl alcohol and aqueous layers will vary depending on the organic content of the soil. Care should be taken to avoid the lower phenol phase and the interphase containing protein and lipid when removing the aqueous upper phase. If a portion of the phenol layer or the interphase is removed, recentrifuge the transferred aqueous phase containing tube to obtain a phase separation that will permit removing the aqueous phase. Alternatively, an equal volume (2 ml) of chloroform may be added to the phenol or interphase contaminated aqueous phase, inverted or vortexed to mix, and the tubes centrifuged at 2,500 x g for 10 minutes at room temperature. Remove the upper aqueous phase and discard the lower chloroform:phenol interphase. Then combine with the rest of the aqueous phase and continue with protocol.

Pellet Resuspension in Solution SR5

Soil types with a high organic content may yield RNA pellets that are difficult to resuspend. Heating the RNA pellet in Solution SR5 at 45°C will aid in the resuspension process. Disrupting the RNA pellet with a pipette tip and vortexing vigorously will also aid in RNA resuspension. It is important to resuspend the pellet completely before applying it to the column in Step 17. Failure to completely resuspend the RNA pellet will result in RNA loss through reduced column binding and will result in reduced column flow rate.

Column Flow

The RNA Capture Columns are rated for gravity flow and should not be used with centrifugal or vacuum force.

Digesting RNA with RNase-Free DNase

NOTE: ONLY RNase-Free DNase may be used with this protocol. The presence of RNases will result in digested RNA.

The presence of carryover DNA with RNA isolated using the RNA PowerSoil™ Total RNA Isolation Kit does not occur with the majority of soil types. It has been noted, however, that soils with high organic matter content may contain carryover DNA co-extracted with the

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isolated RNA. The following protocol, used in conjunction with the manufacturers instructions, should serve as a guide to DNase digesting RNA.

- a. If using the entire RNA sample, add the appropriate amount of DNase buffer, water and up to 4 Units of DNase to the RNA sample to obtain a total volume of 200 μ l. A typical 10X DNase digestion buffer is 10 mM CaCl_2 and 10 mM MgCl_2 in Tris-HCl buffer, pH 7.5.
- b. Incubate at 37°C for 30 to 45 minutes.
- c. Add 200 μ l of phenol:chloroform:isoamyl alcohol (pH 6.5 – 8.0) and vortex to mix. Incubate at room temperature for 5 minutes.
- d. Centrifuge the sample at 10,000 x g for 5 minutes.
- e. Carefully remove the upper aqueous phase and transfer it to another tube.
- f. Add 1/10th volume of 5M NaCl, two volumes of 100% ethanol and invert to mix.
- g. Incubate at -20°C for 30 minutes and centrifuge at 10,000 x g for 10 minutes.
- h. Decant the supernatant and air dry the pellet.

Resuspend the pellet in an appropriate volume of Solution SR7. The RNA can be used directly in an RT-PCR reaction without dilution.

Preparing Phenol:Chloroform:Isoamyl Alcohol

WARNING: Wear gloves, laboratory coat and safety glasses when handling phenol. Phenol:chloroform:isoamyl alcohol is a caustic organic solution. User should review the vendor provided MSDS and accident procedures for this reagent. Do not inhale vapors. Follow local ordinances for disposal of phenol waste. Phenol is highly corrosive and can cause severe burns. Chloroform is a carcinogen. In the event of an accident, seek medical attention immediately.

NOTE: Phenol and phenol:chloroform:isoamyl alcohol are subject to oxidation reactions that cause them to become yellow or pink colored, which serves as an indicator that the phenol is NOT useable for RNA extraction. Using colored phenol or colored phenol:chloroform:isoamyl alcohol will result in quality compromised RNA. Prior to each use, a sample of the phenol:chloroform:isoamyl alcohol should be placed in a clear container and its clarity determined. When not in use store the phenol:chloroform:isoamyl alcohol at 4°C in the dark. Securely cap when not in use and do not expose to light for prolonged periods.

Preparing Phenol:Chloroform:Isoamyl Alcohol Solution

Mix 25 parts purified phenol, 24 parts chloroform, and one part isoamyl alcohol. This solution can be stored under TE buffer or 0.1M Tris, pH 8.0, for periods up to 3 months at 4°C. Store in an amber bottle to protect from light. It is recommended, if storing under TE Buffer, to add a small volume of a Tris buffer to this solution.

NOTE: Chloroform is mixed with phenol to increase the efficiency of nucleic acid extractions by reducing losses of DNA and RNA at the phenol:aqueous interphase. Chloroform

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denatures proteins and aids in the removal of lipids, while isoamyl alcohol reduces foaming during the extraction and facilitates the separation of the aqueous and organic phases.

To make Purified Phenol (a component of final solution)

Commercial, liquefied phenol may be used for nucleic acid extraction without redistillation if the phenol is colorless. Crystalline or liquefied phenol that is yellow or pink is not suitable for RNA isolation and will result in compromised RNA quality. Colored phenol may be redistilled at 160°C with the proper laboratory equipment and safety precautions to remove contaminants that cause breakdown or cross linking of RNA and DNA. Unbuffered liquefied or redistilled phenol should be stored frozen at -15 to -25°C in aliquots until needed. Crystalline phenol may be used if the crystals are white and the phenol buffer is equilibrated as described below:

1. Remove phenol aliquots from the freezer and allow to warm to room temperature.
2. Liquefy phenol by immersing in a water bath at 68°C. (NOTE: 8-hydroxyquinoline may be added to a final concentration of 0.1%. This yellow compound is an antioxidant, a partial inhibitor of RNase, and a weak chelator of metal ions. Because the yellow coloring of the 8-hydroxyquinoline will mask phenol color changes due to oxidation, replace the phenol containing 8-hydroxy-quinoline after approximately 6 months storage.) Alternatively, liquefy crystalline phenol by mixing it with an equal volume of 1.0 M Tris-HCl, pH 8.0, and immersing the container in a water bath at 68°C.
3. Transfer the liquefied phenol to a container that will accept a volume equal to the phenol volume.
4. Add an equal volume of 1.0 M Tris-HCl, pH 8.0, invert to mix and allow to stand until a clearly defined phenol:aqueous interphase forms.
5. Remove a sample of the upper aqueous phase and determine the pH.
6. Transfer the remaining upper aqueous phase into an organic waste container.
7. Repeat steps 4 and 5 until the recovered aqueous pH is less than or equal to 8.0 (NOTE: pH greater than 8.0 will not efficiently remove nucleic acid contaminating substances from soil.).
8. Add an equal volume of 0.1M Tris (pH 8.0), invert to mix and allow to stand until a clearly defined phenol:aqueous interphase forms.
9. Remove the 0.1M Tris (8.0) layer and add an equal volume of TE Buffer.
10. Store the equilibrated phenol for up to 3 months at 2-8°C, protected from light in an amber glass bottle.

Determining the pH of Phenol

Accurate pH measurements of organic phenol and phenol:chloroform can be difficult to achieve. Standard reference electrodes measure the liquid junction potential between the electrode's potassium chloride filling solution and the sample. Organic liquids such as phenol and chloroform have very low dielectric constants compared to water. A very large liquid junction potential, present with phenol, can cause problems such as pH drift, long stabilization times and damage to the pH electrode. Because of this, pH paper has often been used to measure the pH of phenol solutions; however, phenol destroys the indicator chemical of the pH paper, resulting in inaccurate pH measurement.

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To accurately measure the pH of saturated phenol, it is necessary to solublize the phenol in an aqueous medium. The following methods are used to determine pH of phenol solutions:

Phenol:chloroform:isoamyl alcohol: Mix 2 ml of the organic phase with 8 ml of methanol and 10 ml of water. Measure the pH of the entire sample.

Saturated phenols: Mix 2 ml of the organic phase with 5 ml of methanol and 13 ml of water. Measure the pH of the entire sample.

List of Recommended Vendors for Phenol: Chloroform: Isoamyl Alcohol

Vendor Name	Chemical Name	Catalog Number	Volume (ml)
Amresco, Incorporated*	Phenol: Chloroform (pH 6.7/8.0) 25:24:1 premixed with isoamyl alcohol	0883-100	100
Amresco, Incorporated*	Phenol: Chloroform (pH 6.7/8.0) 25:24:1 premixed with isoamyl alcohol	0883-400	400
VWR International**	Phenol: Chloroform premixed with Isoamyl Alcohol 25:24:1	100513-510	100
VWR International**	Phenol: Chloroform Buffered Solution 25:24:1	IB05174	400

*www.amresco-inc.com or (US) 800.829.2802

** www.vwr.com or (US) 800.932.5000

* **International customers should contact their local distributor



Other quality products available from MO BIO Laboratories, Inc.

<u>Kit Description</u>	<u>Catalog Number</u>
RNA Isolation Kits	
Plant RNA Isolation Kit (20 preps)	13300-20
Plant RNA Isolation Kit (50 preps)	13300-50
Tissue RNA Isolation Kit (50 preps)	15000-50
Tissue RNA Isolation K (250 preps)	15000-250
Microbial RNA Isolation Kit (50 preps)	15800-50
Microbial RNA Isolation Kit (250 preps)	15800-250
DNA Isolation Kits	
PowerSoil™ DNA Isolation Kit (50 preps)	12888-50
PowerMax™ Soil DNA Isolation Kit (10 preps)	12988-10
UltraClean™ Soil DNA Kit (50 preps)	12800-50
UltraClean™ Soil DNA Kit (100 preps)	12800-100
UltraClean™ Mega Soil DNA Kit (10 preps)	12900-10
UltraClean-htp™ 96 Well Soil DNA Kit (4 x 96 preps)	12896-4
UltraClean-htp™ 96 Well Soil DNA Kit (12 x 96 preps)	12896-12
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
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

Contact Information

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Ordering Information

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