PowerBiofilm®
RNA Isolation Kit

(For isolation of total RNA from biofilm including microbial mats)

Catalog No.	Quantity
25000-50	50 Preps

Instruction Manual

Please recycle

Version: 11142013

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Introduction

The PowerBiofilm® RNA Isolation Kit is the first of its kind designed for isolating high quality RNA from all types of biofilm samples including microbial mats. Our novel bead tube mix and enhanced lysis buffers help to dissolve polysaccharides to enable lysis of organisms in even the most complex biofilm samples. Our patented Inhibitor Removal Technology® (IRT) is included which allows for inhibitor free, purified RNA that can be used for a multitude of downstream applications. RNase-Free DNase I is provided for on-column genomic DNA removal during the protocol, saving time and post-processing steps.

Protocol Overview

0.05 to 0.20 g of sample material is added to the PowerBiofilm® Bead Tube then heated to activate lysis components that help to dissolve polysaccharides. Lysis continues through vortex mixing using our Vortex Adapter, followed by protein and inhibitor removal to precipitate out humic substances as well as polyphenolics and polysaccharides. Total RNA is captured on the novel MO BIO Laboratories flat bottom silica spin column where an on-column DNase step is incorporated to remove genomic DNA. The column is then washed and the RNA eluted. The purified RNA is ready to use in downstream applications including RT-PCR, qRT-PCR, cDNA synthesis or RNA amplification.

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

Other Related Products	Catalog No.	Quantity
Vortex Adapter for Vortex Genie® 2	13000-V1-24	Holds 24 (2 ml) Tubes
Vortex Genie® 2 Vortex	13111-V-220 13111-V	1 unit (220V) 1 unit (120V)
UltraClean® Lab Cleaner	12095-250 12095-500 12095-1000	250 ml squeeze bottle 500 ml spray bottle 1 liter bottle
RNase-Free Gloves	1556-XS 1556-S 1556-M 1556-L	Bag of 150 Bag of 150 Bag of 150 Bag of 150
PowerBiofilm® DNA Isolation Kit	24000-50	50 preps

PowerBiofilm® RNA Isolation Kit

Prepare Sample

- Add biofilm sample to PowerBiofilm® Bead Tube
- Add Solution BFR1
- Add Solution BFR2

Cell Lysis

- Heat at 65°C
- Attach to Vortex Adapter
- Vortex

Centrifuge

Inhibitor Removal Technology®

- Add Solution BFR3
- Incubate at 4°C

Centrifuge

Bind RNA

- Add Solutions BFR4 and BFR5
- Vortex
- Load into Spin Filter

Centrifuge

DNase I Treatment

- Wash with Solution BFR6
- Add DNase I and Solution BFR7
- Incubate at room temperature

Wash

- Wash with Solution BFR8
- Wash with Solution BFR6
- Wash with Solution BFR5

Centrifuge

Elute

- Elute with Solution BFR9

Equipment Required

Microcentrifuge (13,000 x *g*)
Pipet (volumes required 1.5 µl - 1000 µl)
Vortex-Genie® 2 Vortex (MO BIO Catalog# 13111-V or 13111-V-220)
Vortex Adapter for 2 ml Tubes (MO BIO Catalog# 13000-V1-24)

Components Not Included

β- mercaptoethanol (βME)

Kit Contents

	Kit Catalog # 25000-50	
Component	**Catalog #**	**Amount**
PowerBiofilm® Bead Tubes	25000-50-BT	50
Solution BFR1	25000-50-1	20 ml
Solution BFR2	25000-50-2	6 ml
Solution BFR3	25000-50-3	11 ml
Solution BFR4	25000-50-4	25 ml
Solution BFR5	25000-50-5	2 x 31 ml
Solution BFR6	25000-50-6	3 x 24 ml
Solution BFR7	25000-50-7	2.5 ml
Solution BFR8	25000-50-8	23 ml
Solution BFR9	25000-50-9	5.5 ml
DNase I (RNase-Free)	25000-50-10	1 vial (1500 units)
Spin Filters	25000-50-SF	50
2 ml Collection Tubes	25000-50-T	250

Kit Storage

Remove lyophilized DNase I and store at 4°C. Store all other reagents and kit components at room temperature (15-30°C). DNase I should be stored at 4°C when lyophilized and -20°C after resuspension (DNase is sensitive to physical denaturation. Do not vortex the resuspended DNase).

Precautions

Please wear gloves when using this product. Avoid all skin contact with kit reagents. 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 at www.mobio.com. Reagents labeled flammable should be kept away from open flames and sparks.

WARNING
Solutions BFR5 and BFR6 are flammable.

Wear gloves when handling PowerBiofilm® Bead Tubes.

Important Notes Before Starting

Solution BFR1 must be warmed at 55°C for 5-10 minutes to dissolve precipitates prior to each use. Solution BFR1 should be used while still warm.

Shake to mix Solution BFR6 before use.

Use only PowerBiofilm® Bead Tubes with this kit.

Prepare Solution BFR1 by adding β- mercaptoethanol (βME)

Add 5 µl of β- mercaptoethanol (βME) for every 345 µl of the **Solution BFR1** for all samples to be processed. For each prep, 350 µl of the Solution BFR1/ β- mercaptoethanol (βME) will be needed.

> **Note:** Prepare **Solution BFR1** in smaller aliquots with fresh βME according to the number of samples you need to process that day instead of adding βME to the whole bottle. Use a fume hood when opening βME to avoid exposure to the chemical.

DNase I Preparation and Storage

To prepare **DNase I stock solution** add RNase-Free water (**Solution BFR9**) to the lyophilized **DNase I** according to the table below and mix gently. Aliquot the enzyme in 50 µl portions and store at -20°C for long term storage. **Note:** The enzyme can be freeze/thawed up to three times without loss of activity.

Catalog#	# of Preps	Units of DNase	Volume of water to resuspend DNase
25000-50	50	1500 U	300 ul

To prepare the **DNase I Solution**, thaw the volume of enzyme needed according to the number of samples. Per prep, combine **5 µl of DNase I enzyme** with **45 µl of Solution BFR7**.

Experienced User Protocol

Please wear gloves at all times

Warm Solution BFR1 prior to use at 55°C for 5-10 minutes. Use Solution BFR1 while still warm. Add βME to Solution BFR1 before use. See Important Notes before starting.

1. Weigh out **0.05 to 0.20 g** of biofilm material and place it into a 2 ml Collection Tube (provided). Centrifuge at 13,000 x *g* for 1 minute. Remove excess liquid using a pipette tip. For less saturated samples (ex. microbial mats) add directly to the PowerBiofilm® Bead Tube **(For information on selecting the right amount of material to add, see Amount of Starting Material in the Hints and Troubleshooting Guide before continuing).**
   Note: Use only PowerBiofilm® Bead Tubes with this kit.
2. Resuspend the biofilm material in **350 µl of Solution BFR1/β- mercaptoethanol (βME**) and transfer to the PowerBiofilm® Bead Tube. For less saturated samples, add **350 µl of Solution BFR1/β- mercaptoethanol (βME**) directly to the PowerBiofilm® Bead Tube already containing the biofilm material.
   Note: Solution BFR1 must be warmed to dissolve precipitates prior to use. Solution BFR1 should be used while still warm. Add βME to Solution BFR1 before use. See Important Notes before starting.
3. Add **100 µl of Solution BFR2**. Vortex briefly to mix
4. Incubate the PowerBiofilm® Bead Tube at 65°C for 5 minutes.
5. Secure the PowerBiofilm® Bead Tube horizontally to a MO BIO Vortex Adapter.
6. Vortex at maximum speed for 10 minutes.
   Note: If you are using the 24 place Vortex Adapter for more than 12 preps, increase the time by 5 – 10 minutes.
7. Centrifuge the tubes at 13,000 x *g* for 1 minute at room temperature.
   CAUTION: Be sure not to exceed 13,000 x *g* or tubes may break.
8. Transfer the supernatant to a clean 2 ml Collection Tube (provided).
   Note: Expect approximately 400 - 450 µl of supernatant depending on sample material. If the volume falls below this range, use less starting material.
9. Add **100 µl of Solution BFR3** and vortex briefly to mix. Incubate at 4°C for 5 minutes.
   Note: Use 200 µl of Solution BFR3 if the sample is known to contain excessive amounts of inhibitors or the supernatant is very darkly colored. See "cDNA Does Not Amplify..." in the Hints and Troubleshooting Guide before continuing.
10. Centrifuge the tubes at 13,000 x *g* for 1 minute at room temperature.
11. Avoiding the pellet, transfer the entire volume of supernatant to a clean 2 ml Collection Tube (provided).
    Note: Expect approximately 375 - 450 µl in volume depending on sample material.
12. Add **450 µl of Solution BFR4** and **450 µl of Solution BFR5** vortex briefly to mix.
13. Load 650 µl of supernatant onto a Spin Filter and centrifuge at 13,000 x *g* for 1 minute. Discard the flow through and repeat until all the supernatant has been loaded onto the Spin Filter.
    Note: A total of two loads for each sample processed are required.
14. Shake to mix Solution BFR6 before use. Add **650 µl of Solution BFR6** and centrifuge at 13,000 x *g* for 1 minute.
15. Discard the flow through and centrifuge again at 13,000 x *g* for 1 minute to remove residual wash.
16. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).
17. To the center of the Spin Filter, add **50 µl of DNase I Solution** (prepared by mixing **45 µl of Solution BFR7** and **DNase I stock solution.** See **Important Notes Before Starting** section).

18. Incubate at room temperature for 15 minutes.
    Note: Do not centrifuge the Spin Filter before the addition of Solution BFR8.
19. Add **400 µl Solution BFR8** and centrifuge the column at 13,000 x *g* for 1 minute.
20. Discard the flow through and add **650 µl of Solution BFR6** and centrifuge at 13,000 x *g* for 1 minute.
21. Discard the flow through and add **650 µl of Solution BFR5** and centrifuge at 13,000 x *g* for 1 minute.
22. Discard the flow through and centrifuge again at 13,000 x *g* for 2 minutes to remove residual wash.
23. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).
24. Add **100 µl of Solution BFR9** to the center of the white filter membrane.
    Note: Eluting with 100 µl of Solution BFR9 will maximize RNA yield. For more concentrated RNA, a minimum of 50 µl of Solution BFR9 can be used. Do not use less than 50 µl of Solution BFR9.
25. Centrifuge at 13,000 x *g* for 1 minute.
26. Discard the Spin Filter basket. The RNA is now ready for any downstream application. No further steps are required. The RNA in the tube can be stored at -80°C until ready for use.

Thank you for choosing the PowerBiofilm® RNA Isolation Kit!

Detailed Protocol

Please wear gloves at all times

Warm Solution BFR1 prior to use at 55°C for 5-10 minutes. Use Solution BFR1 while still warm. Add βME to Solution BFR1 before use. See Important Notes before starting.

1. Weigh out **0.05 to 0.20 g** of biofilm material and place it into a 2 ml Collection Tube (provided). Centrifuge at 13,000 x *g* for 1 minute. Remove excess liquid using a pipette tip. For less saturated samples (ex. microbial mats) add directly to the PowerBiofilm® Bead Tube **(For information on selecting the right amount of material to add, see Amount of Starting Material in the Hints and Troubleshooting Guide before continuing).**

   Note: Use only PowerBiofilm® Bead Tubes with this kit.

   What's happening: Biofilm samples will vary in their moisture content. It is important to remove residual liquid to prevent dilution of the lysis components which could result in reduced RNA yield. Some biofilm samples, such as microbial mats may be added directly to the PowerBiofilm® Bead Tube without an initial centrifugation step.

2. Resuspend the biofilm material in **350 µl of Solution BFR1/β- mercaptoethanol (βME**) and transfer to the PowerBiofilm® Bead Tube. For less saturated samples, add **350 µl of Solution BFR1/β- mercaptoethanol (βME**) directly to the PowerBiofilm® Bead Tube already containing the biofilm material.

   Note: Solution BFR1 must be warmed to dissolve precipitates prior to use. Solution BFR1 should be used while still warm. Add βME to Solution BFR1 before use. See Important Notes Before Starting.

   What's happening: Solution BFR1 is a component of patented Inhibitor Removal Technology® (IRT). It is a strong lysing reagent that includes a detergent to help break cell walls and stabilizes and protects RNA from degradation. When cold, this solution will form a white precipitate in the bottle. Heating to 55°C will dissolve the components without harm. Solution BFR1 can be used while it is still warm.

3. Add **100 µl of Solution BFR2**. Vortex briefly to mix.

   What's happening: Solution BFR2 contains a chaotropic agent that aids in lysis. BFR2 also stabilizes and protects RNA integrity.

4. Incubate the PowerBiofilm® Bead Tube at 65°C for 5 minutes.

   What's happening: Lysis components are heat activated to aid in the breakdown of extracellular polymeric substances (EPS).

5. Secure the PowerBiofilm® Bead Tube horizontally to a MO BIO Vortex Adapter.

6. Vortex at maximum speed for 10 minutes.

   Note: If you are using the 24 place Vortex Adapter for more than 12 preps, increase the time by 5 – 10 minutes.

What is happening: Dissolution of the biofilm matrix and lysis of microbial cells occurs using a combination of chemical (lysis buffers) and mechanical (bead beating) lysis conditions. Use of the vortex adapter will maximize homogenization by holding the tubes equal distance and angle from the center of rotation. Avoid using tape, which can become loose and result in reduced homogenization efficiency.

7. Centrifuge the tubes at 13,000 x *g* for 1 minute at room temperature.
   CAUTION: Be sure not to exceed 13,000 x *g* or tubes may break.

   What is happening: Cell debris is pelleted along the side of the tube while the RNA remains in the supernatant. This step is important for the removal of contaminating non-RNA organic and inorganic matter that may reduce the RNA purity and inhibit downstream applications.

8. Transfer the supernatant to a clean 2 ml Collection Tube (provided).

   Note: Expect approximately 400 - 450 µl of supernatant depending on sample material. If the volume falls below this range, use less starting material.

9. Add **100 µl of Solution BFR3** and vortex briefly to mix. Incubate at 4°C for 5 minutes.

   Note: Use 200 µl of Solution BFR3 if the sample is known to contain excessive amounts of inhibitors or the supernatant is very darkly colored. See "cDNA Does Not Amplify..." in the Hints and Troubleshooting Guide before continuing.

   What's happening: Solution BFR3 is a component of patented Inhibitor Removal Technology® (IRT) and is a second reagent to remove additional organic and inorganic material including humic acid, cell debris, polyphenolics, polysaccharides and proteins. The system works by using changes in pH to precipitate the insoluble large macromolecules. The nucleic acids do not precipitate and are cleared of inhibitors. It is important to remove contaminating organic and inorganic matter that may reduce the RNA purity and inhibit downstream RNA applications.

10. Centrifuge the tubes at 13,000 x *g* for 1 minute at room temperature.

11. Avoiding the pellet, transfer the entire volume of supernatant to a clean 2 ml Collection Tube (provided).

    Note: Expect approximately 375 - 450 µl in volume depending on sample material.

    What's happening: The pellet at this point contains additional non-RNA organic and inorganic material. For best RNA yields and quality, avoid transferring any of the pellet.

12. Add **450 µl of Solution BFR4** and **450 µl of Solution BFR5** vortex briefly to mix.

    What's happening: Solution BFR4 is a high concentration salt solution and solution BFR5 is ethanol. Both components are necessary to create the conditions required for efficient binding of the RNA to the spin column while allowing proteins and cellular debris to pass through.

13. Load 650 µl of supernatant onto a Spin Filter and centrifuge at 13,000 x *g* for 1 minute. Discard the flow through and repeat until all the supernatant has been loaded onto the Spin Filter.

    Note: A total of two loads for each sample processed are required.

    What's happening: RNA is selectively bound to the silica membrane in the Spin Filter basket and the flow through containing non-RNA components is discarded.

14. Shake to mix Solution BFR6 before use. Add **650 µl of Solution BFR6** and centrifuge at 13,000 x *g* for 1 minute.

    What's happening: Solution BFR6 is an alcohol based wash solution used to wash the spin filter column in preparation for the on column DNase I digestion. This wash solution removes residual salt and other contaminants while allowing the RNA to stay bound to the silica membrane.

15. Discard the flow through and centrifuge again at 13,000 x *g* for 1 minute to remove residual wash.

    What's happening: Complete removal of Solution BFR6 is required for efficient and complete DNase I digestion.

16. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).

17. To the center of the Spin Filter, add **50 µl of DNase I Solution** (prepared by mixing **45 µl of Solution BFR7** and **DNase I stock solution.** See **Important Notes Before Starting** section).

18. Incubate at room temperature for 15 minutes.

    Note: Do not centrifuge the Spin Filter before the addition of Solution BFR8.

    What's happening: DNase I is mixed with high activity digestion buffer and is used to completely remove genomic DNA from the Spin Filter membrane. If the RNA is to be used for reverse transcription and or RT PCR, it is highly recommended to remove all genomic DNA with a DNase I digestion.

19. Add **400 µl Solution BFR8** and centrifuge the column at 13,000 x *g* for 1 minute.

    What's happening: Solution BFR8 is a wash buffer used to inactivate DNase I and wash away residual enzyme and digested DNA while allowing RNA to remain tightly bound to the spin column.

20. Discard the flow through and add **650 µl of Solution BFR6** and centrifuge at 13,000 x *g* for 1 minute.

    What's happening: Solution BFR6 is an ethanol based wash buffer used to remove residual salt and contaminants on the column in preparation for the release and elution of the bound RNA. Complete removal of all traces of the wash solution is critical.

21. Discard the flow through and add **650 µl of Solution BFR5** and centrifuge at 13,000 x *g* for 1 minute.

    What's happening: Solution BFR5 ensures complete removal of Solution BFR6 which will result in higher RNA purity and yield.

22. Discard the flow through and centrifuge again at 13,000 x *g* for 2 minutes to remove residual wash.

    What's happening: The second spin removes residual Solution BFR5. It is critical to remove all traces of wash solution because the ethanol in Solution BFR5 can interfere with downstream RNA applications.

23. Place the Spin Filter basket into a clean 2 ml Collection Tube (provided).

24. Add **100 µl of Solution BFR9** to the center of the white filter membrane.

    Note: Eluting with 100 µl of Solution BFR9 will maximize RNA yield. For more concentrated RNA, a minimum of 50 µl of Solution BFR9 can be used. Do not use less than 50 µl of Solution BFR9.

    What's happening: Solution BFR9 is highly pure water used to elute the RNA from the silica membrane of the spin column. Placing Solution BFR9 (sterile elution buffer) in the center of the small white membrane will make sure the entire membrane is wetted. This will result in a more efficient and complete release of the RNA from the silica Spin Filter membrane. As Solution BFR9 passes through the silica membrane, RNA that was bound in the presence of high salt is selectively released by Solution BFR9 which lacks salt.

25. Centrifuge at 13,000 x *g* for 1 minute.

26. Discard the Spin Filter basket. The RNA is now ready for any downstream application. No further steps are required. The RNA in the tube can be stored at -80°C until ready for use.

Thank you for choosing the PowerBiofilm® RNA Isolation Kit!

Hints and Troubleshooting Guide

Amount of Starting Material
This kit is designed to process 0.05 to 0.2 g of biofilm or microbial mat material. The actual amount to use will depend on the type of biofilm and microbial density. If supernatant amounts fall under the range provided in Step 8 of the protocol then RNA yields will not be optimal and less sample material should be used for processing. A recommended starting amount is 0.1 - 0.15 g. For examples of expected yields, see the table under "Expected RNA Yields" below.

Forgetting to Warm Solution BFR1
If BFR1 is not warmed prior to use, continue with the protocol. You will still obtain RNA, but the yields may not be optimal.

Expected RNA Yields
RNA yields will vary depending on the type of biofilm. Yields may also vary between samples of the same biofilm due to their structure. Examples of expected yields are provided as a reference. Due to diversity of biofilm sample types, yields may fall outside of the examples provided.

Biofilm Type	Sample Amount (g)	RNA Yield (ng/µl)
Lagoon Rocks	0.2 - 0.23	37 - 60
Phototrophic Biofilm (Microbial Mat)	0.10 - 0.15	10 - 100
Button Thrombolites (Microbial Mat)	0.25	1 - 1.7
Deep Sea Microbial Mat	0.20 - 0.30	11 - 35

Low or No RNA Yield
Yields may be significantly reduced if too much starting material is used or the PowerBiofilm® Bead Tubes are not used. To avoid sample loss:

- Do not use more sample than the specified range (0.05 – 0.20 g).
- Do not use any other bead tube except the ones provided in this kit. The PowerBiofilm® Bead Tubes have been specially designed for use in this kit.

cDNA Does Not Amplify or Has Reduced Amplification Efficiency
Biofilms with high concentrations of humic substances and other contaminants may yield RNA with some inhibitor carryover, which can prevent target sequences from amplifying in RT-PCR. Under such circumstances, the template cDNA can be diluted one to several fold for successful PCR. For additional preps of the same or similar sample type, use 200 µl of BFR3 at step 9 to eliminate inhibitor carry over.

RNA Appears Degraded on Agarose Gels
The use of Beta mercaptoethanol (βME) will destroy RNases and should be added fresh to Solution BFR1. If RNA still appears degraded, the problem may be caused by the following:

- Make sure that biofilm samples are fresh and stored at 4°C or -20°C if not processed immediately. Storage at room temperature will cause considerable RNA degradation and loss.
- Prepare Solution BFR1 in smaller aliquots with fresh βME according to the number of samples you need to process that day instead of adding βME to the whole bottle.

Hints and Troubleshooting Guide cont.

- RNA will not always run correctly on non-denaturing gels and may appear smeared due to RNA secondary structure. Run RNA on a denaturing gel according to the "**Protocol for Formaldehyde Gel Electrophoresis**".
- The 260/280 ratio is a good indicator of RNA quality as the absorbance at 260 will increase as RNA is digested into smaller fragments and single nucleotides. A ratio above 2.3 may indicate RNA degradation.

RNA Floats Out of Well When Loaded on a Gel

Residual Solution BFR5 may be in the final sample. To ensure complete drying of the spin filter membrane, centrifuge the spin filter in a clean 2 ml Collection Tube for an additional minute.

- Ethanol precipitation is the best way to remove residual Solution BFR5. (See "Concentrating the RNA" below.)
- If you live in a humid climate, you may experience increased difficulty with drying of the membrane in the centrifuge. Increase the centrifugation time at step 22 by another minute.

RNA has Low $A_{260/280}$ Ratio

The ratio for pure RNA should be1.9-2.1. $A_{260/280}$ reading below 1.6 may have significant protein contamination.

- Make sure that the BFR8 wash was performed after the DNase I treatment.
- A low ratio may also occur when the sample is measured by UV spectrophotometry in water. The low pH of water can influence the 280 reading and cause reduced sensitivity to protein contamination*. Re-measure the 260/280 diluting the RNA for measurement in 10 mM Tris pH 7.5.

**Wilfinger, W.W., Mackey, M., and Chomczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques 22, 474.*

Genomic DNA Contamination in the RNA

The PowerBiofilm® RNA Isolation Kit is provided with high quality RNase-Free DNase I for on-column digest. When used with the Solution BFR7 included in the kit, activity of the DNase I will be optimal for on-column digestion.

- Use only the buffer provided with the DNase I for on-column digest.
- Make sure to perform the digest for the 15 minutes as recommended. Shortening the digest time may result in incomplete genomic DNA removal. RNA will not be degraded during this incubation. You may extend the DNase I digest up to 30 minutes.

Concentrating the RNA

Your final volume will be 50 µl - 100 µl. If this is too dilute for your purposes, add 5 µl of 3M Sodium Acetate and mix. Then add 2 volumes of 100% cold ethanol. Mix, and incubate at -70°C for 15 minutes or -20°C for 2 hours to overnight. Centrifuge at 10,000 x *g* for 10-15 minutes at 4°C. Decant all liquid. Briefly dry residual ethanol in a speed vac or ambient air. Avoid over-drying the pellet or resuspension may be difficult. Resuspend precipitated RNA in desired volume of RNase-Free water (Solution BFR9).

Storing RNA

RNA is eluted in RNase-Free water (Solution BFR9) and should be used immediately or stored at -20°C or -80°C to avoid degradation. RNA can be precipitated in EtOH and stored at -20°C to ensure minimal degradation during long term storage.

Technical Guide

Protocol for Formaldehyde Agarose Gel Electrophoresis
Solutions needed.

10x Formaldehyde agarose gel buffer
200 mM 3-[N-morpholino] propanesulfonic acid (MOPS) (free acid)
50 mM Sodium Acetate
10 mM EDTA
pH to 7.0 with Sodium Hydroxide.

1x Formaldehyde agarose gel buffer (1L)
100 ml 10x Formaldehyde Agarose gel buffer
20 ml 37% (12.3 M) Formaldehyde
880 ml DEPC treated water

5x RNA Loading Dye
16 µl Saturated aqueous Bromophenol blue solution
80 µl .5 M EDTA, pH 8.0
720 µl 37% (12.3 M) Formaldehyde
2 ml 100% Glycerol
3084 µl Formamide
4 ml 10x Formaldehyde agarose gel buffer

Formaldehyde Agarose Gel preparation 1.2% in 100 ml
Mix the following:
1.2 g Agarose
10 ml 10x Formaldehyde agarose gel buffer
90 ml DEPC treated water

Heat the mixture in a microwave oven to melt the agarose. Cool to 65°C in a waterbath. Add 1.8 ml 37% (12.3 M) Formaldehyde and 2 µl of 5 mg/ml Ethidium Bromide. Swirl to mix and pour into a gel box. The gel must be pre-ran for 30 minutes in 1x Formaldehyde Agarose gel buffer before loading the samples.

RNA Sample Preparation for Formaldehyde Gels
The eluted RNA samples must be denatured before running on a formaldehyde agarose gel. To the sample, add 1 volume of 5x RNA loading dye for each 4 volumes of RNA sample (i.e. 2 µl of 5x RNA loading dye for each 8 µl of RNA sample).

Mix the samples and briefly centrifuge to collect the sample at the bottom of the tube.

Incubate at 65°C for 3-5 minutes, then chill on ice and load in the Formaldehyde agarose gel. Run the gel at 5-7 V/cm in 1x Formaldehyde Agarose gel buffer.

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

Contact Information

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

Products recommended for you

For a complete list of products available from MO BIO Laboratories, Inc., visit www.mobio.com

Description	Catalog No.	Quantity
PowerBiofilm® DNA Isolation Kit	24000-50	50 preps
PowerPlant® Pro DNA Isolation Kit	13400-50	50 preps
PowerPlant® RNA Isolation Kit	13500-50	50 preps
PowerPlant® RNA Isolation Kit with DNase	13550-50	50 preps
Vortex Genie® 2 Vortex	13111-V 13111-V-220	1 unit (120V) 1 unit (220V)
Vortex Adapter for Vortex Genie® 2	13000-V1-24	Holds 24 (2 ml) Tubes
RTS™ DNase Kit	15200-50	50 preps