



BiOstic™ Stabilized Blood RNA Isolation Kit

(For use with PAXgene™ Blood RNA Tubes*)

| Catalog No. | Quantity |
|-------------|----------|
| 12231-50 | 50 Preps |

Instruction Manual



Please recycle

Version: 11092011

*PAXgene™ is a trademark of Qiagen. PAXgene™ Blood RNA Tubes are not included with this kit. MO BIO Laboratories, Inc. is not affiliated with the manufacturer of PAXgene™ tubes in any way.

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



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Introduction

The BiOstic™ Stabilized Blood RNA Isolation Kit provides a way to purify RNA from blood samples collected in PAXgene™ Blood RNA Tubes. The PAXgene™ Blood RNA Tube allows for collection of 2.5 ml of blood directly into a stabilization reagent. For details on the correct procedure for collecting blood in PAXgene™ Blood RNA tubes, please refer to the RNA Tube Product Circular at www.preanalytix.com.

Protocol Overview

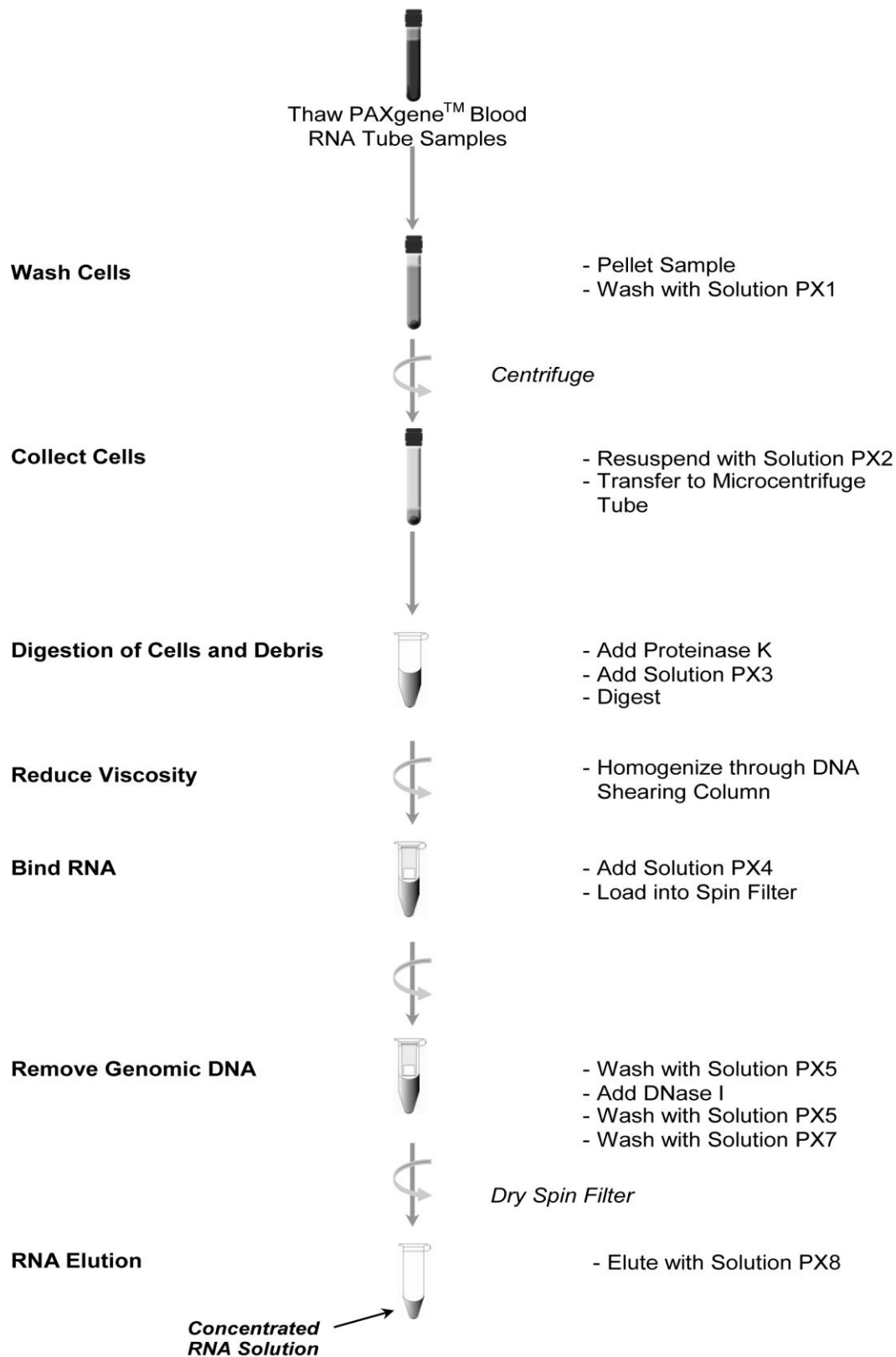
BiOstic™ silica spin filter column products utilize the novel MO BIO Laboratories flat bottom spin filter design, which provides improved sample processing and yields. The bucket configuration allows for enhanced sample flow through and increased membrane drying after wash steps since the entire membrane is accessible to air flow. Silica technology provides a robust and fast way to purify nucleic acids without the use of organic solvents or cesium chloride gradients.

To use the BiOstic™ Stabilized Blood RNA Isolation Kit, samples collected in PAXgene™ Blood RNA Tubes are thawed at room temperature and the cells are pelleted with centrifugation. Samples are washed with a specially formulated buffer and lysed in a chaotropic salt buffer and a room temperature stable proteinase K followed by complete homogenization using carbide beads supported in a DNA Shearing column. Thorough homogenization allows for increased recovery of the RNA. The RNA is bound to a silica spin filter column and residual DNA is removed with an on-column DNase I digest. The Spin filter column is washed to remove protein and salts and the final RNA is eluted ready to use in downstream applications.

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

| Other Related Products | Catalog No. | Quantity |
|--|-------------|-----------------------|
| UltraClean™ Lab Cleaner | 12095-250 | 250 ml squeeze bottle |
| | 12095-500 | 500 ml spray bottle |
| | 12095-1000 | 1 liter bottle |
| RNase-Free Gloves | 1555-XS | Bag of 100 |
| | 1555-S | Bag of 100 |
| | 1555-M | Bag of 100 |
| | 1555-L | Bag of 100 |
| BiOstic™ Blood Total RNA Isolation Kit | 12230-50 | 50 preps |

BiOstic™ Stabilized Blood RNA Isolation Kit





Equipment Required

Microcentrifuge ($\geq 16,000 \times g$)
Centrifuge for 15 ml tubes ($2500 \times g$)
Pipettors
Waterbath or heat block set at 55°C
Vortex

Reagents Required but not Included

PAXgene™ Blood RNA Tubes
 β - mercaptoethanol (β ME)

Kit Contents

| Component | Kit Catalog# 12231-50 | |
|------------------------------------|-----------------------|------------------------|
| | Catalog# | Amount |
| Solution PX1 | 12231-50-1 | 220 ml |
| Solution PX2 | 12231-50-2 | 20 ml |
| Solution PX3 | 12231-50-3 | 20 ml |
| Solution PX4 | 12231-50-4 | 20 ml |
| Solution PX5 | 12231-50-5 | 46 ml |
| Solution PX6 | 12231-50-6 | 3 ml |
| Solution PX7 | 12231-50-7 | 2 x 28 ml |
| Solution PX8 | 12231-50-8 | 6 ml |
| DNase I (RNase-Free) | 12231-50-9 | 1 vial (1500 units) |
| Proteinase K Solution (20mg/ml) | 12231-50-10 | 2.25 ml |
| Carbide Bead Tubes | 12231-50-BT | 50 |
| Spin Filters (blue) | 12231-50-SF | 50 |
| 2 ml Collection Tubes | 12231-50-T1 | 200 |
| DNA Shearing Columns (clear) | 12231-50-T2 | 50 |

Kit Storage

Remove lyophilized DNase I and store at 4°C . Proteinase K is stable at room temperature. For prolonged storage, place the Proteinase K at 4°C . Store all other reagents and kit components at room temperature ($15\text{-}30^{\circ}\text{C}$). **Note:** After resuspension, store DNase I at -20°C (DNase I is sensitive to physical denaturation. Do not vortex the resuspended DNase I).

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 PX4 and PX7 contain ethanol. They are flammable.



Important Notes Before Starting

This protocol describes how to extract total RNA from 2.5 ml of whole blood collected in the PAXgene™ Blood RNA Tube.

For the PAXgene™ Blood RNA Tube:

- PAXgene™ Blood RNA Tubes must be incubated for a minimum of two hours before refrigerating or freezing. If the sample is frozen before this incubation is performed, allow the two hour incubation to proceed upon completely thawing of the samples to room temperature.
- The PAXgene™ Blood RNA Tube will stabilize cellular RNA at room temperature for up to 72 hours, up to 5 days at 4°C, or up to 6 months at -20°C or -80°C.
- Do not freeze PAXgene™ Blood RNA Tubes in a Styrofoam tray. Samples must be frozen in a wire rack and in an upright position.
- If the sample is to be stored at -80°C, freeze the tubes first at -20°C for 24 hours before transferring to a -80°C freezer.
- Allow PAXgene™ Blood RNA Tubes to thaw at ambient temperature for approximately two hours. Do not thaw tubes at elevated temperatures.
- Before processing the thawed sample, mix the PAXgene™ Blood RNA Tubes by inversion 10 times before using.

For the RNA extraction:

- For each prep you will need four 2 ml Collection Tubes, 1 Spin Filter (blue), 1 DNA Shearing Column (clear), and 1 Carbide Bead Tube. To speed processing, in advance, label the caps of the tubes and columns to make Spin Filter transfer more convenient.
- Prepare the DNA Shearing Column by placing the empty column into one of the four labeled 2 ml Collection Tubes from above. Pour the contents of the Carbide Bead Tube into the column. Close and label the cap.
- All steps in the protocol are carried out at room temperature.
- A precipitate may form in Solution PX3 upon storage. If necessary, dissolve any precipitates by warming the solution at 37°C for 10 minutes.
- Add 10 µl of β- mercaptoethanol (βME) for every 1 ml of Solution PX3 for all samples to be processed. You will use 300 µl of Solution PX3 per prep.

Note: Prepare Solution PX3 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. Solution PX3 containing βME is stable at room temperature for one month.

DNase I Stock Enzyme and DNase I Solution Preparation and Storage:

Prepare **DNase I stock enzyme** by adding RNase-Free water (**Solution PX8**) to the lyophilized DNase I according to the table below and mix gently. Aliquot the DNase I stock enzyme in 50 µl portions and store at -20° C for long term storage. Note: The DNase I stock enzyme can be freeze/thawed up to three times without loss of activity.

| Cat# | # of Preps | Units of DNase I | Volume of water to resuspend DNase I |
|-----------|------------|------------------|--------------------------------------|
| 12231-20 | 20 | 750 U | 150 µl |
| 12231-50 | 50 | 1500 U | 300 µl |
| 12231-100 | 100 | 2500 U | 550 µl |

Prepare the **DNase I Solution**, by thawing the volume of DNase I stock enzyme needed according to the number of samples. Per prep, combine **5 µl of DNase I enzyme** with **45 µl of Solution PX6**.



Experienced User Protocol

Please wear gloves at all times

PAXgene™ Tube Sample Lysis and Binding to the Spin Filter:

1. Remove PAXgene™ Blood RNA Tubes from the freezer and allow samples to thaw at room temperature for two hours. Invert to mix 10 times before starting.
2. Centrifuge blood tubes at 2500 x *g* for 10 minutes to pellet the cells. Decant the supernatant.
3. Wash the cell pellet by adding **4 ml of Solution PX1** to the tube and vortex for 30 seconds at high speed to resuspend as much of the pellet as possible.
4. Centrifuge at 2500 x *g* for 10 minutes to pellet the cells. Decant the supernatant and allow the tube to drain upside down on a paper towel to remove as much liquid as possible. Tap the tube on the paper towel to completely drain.
5. Add **300 µl of Solution PX2** to the pellet in the PAXgene™ Blood RNA Tube and vortex for 1-2 minutes at high speed to thoroughly resuspend the pellet. Transfer the lysate to a new 2 ml Collection Tube (provided).
6. Add **40 µl of Proteinase K (20 mg/ml)** and **300 µl of Solution PX3**. **Note:** See Important Notes Before Starting on the previous page for preparation of Solution PX3.
Note: Do not pre-mix the Proteinase K and Solution PX3 together before adding to the resuspended pellet.
7. Vortex at high speed for 10 seconds and incubate at 55°C for **10 minutes**. Agitate the sample with vortexing several times during the incubation. Alternatively, a shaking water bath may be used.
8. For each prep, prepare a **DNA Shearing Column**. Place an empty DNA Shearing Column (clear) into a new 2 ml Collection Tube (provided) and pour into the Column the contents of a **Carbide Bead Tube**.
9. Briefly pulse centrifuge the lysate from step 7 to bring the liquids down from the lid. Transfer the digested lysate to the **DNA Shearing Column** containing carbide beads.
10. Centrifuge the tubes at full speed ($\geq 16,000 \times g$) for 3 minutes.
11. Discard the DNA Shearing Spin Basket and add **350 µl of Solution PX4** to the sample flow-through. Mix thoroughly with vortexing.
12. Transfer 600 µl of sample onto the **Spin Filter (blue)**. Centrifuge at 10,000 x *g* for 1 minute to bind. Discard the flow through and put the **Spin Filter** back into the **2 ml Collection Tube**. Load the remaining lysate into the **Spin Filter** column and centrifuge at 10,000 x *g* for 1 minute to bind. Discard the flow-through and put the **Spin Filter** back into the **2 ml Collection Tube**.
13. Add **400 µl of Solution PX5** to the **Spin Filter** column. Centrifuge at 10,000 x *g* for 1 minute. Discard the flow through and place the **Spin Filter** column into the same **2 ml Collection Tube** and centrifuge at 10,000 x *g* for an additional 2 minutes. This step will ensure the complete removal of Solution PX5 before the DNase I treatment.
Note: Incomplete removal of Solution PX5 may inhibit the DNase I activity.

DNase I Treatment:

14. Transfer the **Spin Filter** to a new **2 ml Collection Tube** (provided). To the center of the Spin Filter, add **50 µl of DNase I Solution** (a mixture of **45 µl of Solution PX6** and **5 µl of DNase I, see Important Notes Before Starting section**). Incubate at room temperature for 15 minutes.
Note: Do not centrifuge the Spin Filter before the addition of Solution PX5 in step 15 below.
15. To the **Spin Filter** column add **400 µl Solution PX5** and centrifuge at 10,000 x *g* for 1 minute. Discard the flow through and place the **Spin Filter** back into the **2 ml Collection Tube**.



Final Washes:

16. Add **500 µl of Solution PX7** to the **Spin Filter**. Centrifuge at 10,000 x *g* for 1 minute. Discard the flow-through and place the **Spin Filter** back into the **2 ml Collection Tube**.
17. Repeat the wash by adding another **500 µl of Solution PX7** to the **Spin Filter**. Centrifuge at 10,000 x *g* for 1 minute. Discard the flow-through and place the **Spin Filter** back into the **2 ml Collection Tube**. Centrifuge the **Spin Filter** for 2 minutes at 10,000 x *g* to completely dry the membrane.

RNA Elution:

18. Transfer the **Spin Filter** column to a new **2 ml Collection Tube** (provided). Add **50 µl Solution PX8 (Sterile Water)** to the center of the Spin Filter membrane. Incubate 1 minute at room temperature then centrifuge the **Spin Filter** at 10,000 x *g* for 1 minute to elute.

NOTE: Placing Solution PX8 in the center of the small white membrane of the Spin Filter will ensure that the entire membrane is completely wetted. This will result in a more efficient and complete release of the RNA from the silica membrane of the Spin Filter.

19. Repeat the elution step by adding another **50 µl of PX8** to the Spin Filter membrane. Centrifuge at 10,000 x *g* for 1 minute.
20. Remove the **Spin Filter** and close the cap of the **2 ml Collection Tube**. The RNA can be stored at -80°C until ready for use.

Thank you for choosing the BiOstic™ Stabilized Blood RNA Isolation Kit.



Detailed Protocol (Describes what is happening at each step)

Please wear gloves at all times

PAXgene™ Tube Sample Lysis and Binding to the Spin Filter:

1. Remove PAXgene™ Blood RNA Tubes from the freezer and allow samples to thaw at room temperature for two hours. Invert to mix 10 times before starting.

What's happening: The PAXgene™ Blood RNA Tubes need to thaw completely at room temperature before use and must have been incubated at least 2 hours at room temperature either before or after freezing.

2. Centrifuge blood tubes at 2500 x g for 10 minutes to pellet the cells. Decant the supernatant.

What's happening: The RBCs and WBCs are collected in the bottom of the blood tube and the stabilization reagent is poured off.

3. Wash the cell pellet by adding **4 ml of Solution PX1** to the tube and vortex for 30 seconds at high speed to resuspend as much of the pellet as possible.

What's happening: The stabilization reagent is washed away to prepare for the lysis steps.

4. Centrifuge at 2500 x g for 10 minutes to pellet the cells. Decant the supernatant and allow the tube to drain upside down on a paper towel to remove as much liquid as possible. Tap the tubes on the paper towel to completely drain.

What's happening: Removal of the stabilization reagent before lysis is important for Proteinase K activity and for binding conditions.

5. Add **300 µl of Solution PX2** to the pellet and vortex for 1-2 minutes at high speed to thoroughly resuspend the pellet. Transfer the lysate to a new 2 ml Collection Tube (provided).

What's happening: Solution PX2 will resolubilize the pellet to make digestion more efficient and allow for strong Proteinase K activity.

6. Add **40 µl of Proteinase K (20 mg/ml)** and **300 µl of Solution PX3**. **Note:** See Important Notes Before Starting for preparation of Solution PX3.

What's happening: The Proteinase K and Solution PX3 will lyse the cells and dissolve the protein from the RBCs packed into the pellet so the lysate will not clog the column.

Note: Do not pre-mix the Proteinase K and Solution PX3 together before adding to the resuspended pellet.

What's happening: Solution PX3 undiluted in Solution PX2 may decrease the activity of Proteinase K.

7. Vortex at high speed for 10 seconds and incubate at 55°C for **10 minutes**. Agitate the sample with vortexing several times during the incubation. Alternatively, a shaking waterbath may be used.

What's happening: Vortexing during the incubation will help resolubilize all the protein and increase Proteinase K access to all of the particulates in the sample. This is important for decreasing the viscosity of the lysate before binding and releasing the RNA.

8. For each prep, prepare a **DNA Shearing Column**. Place an empty DNA Shearing Column (clear) into a new 2 ml Collection Tube (provided) and pour into the Column the contents of a **Carbide Bead Tube**.
9. Briefly pulse centrifuge the lysate from step 7 to bring the liquids down from the lid. Transfer the digested lysate to the **DNA Shearing Column** containing carbide beads.

What's happening: Carbide beads are for shearing the genomic DNA and help prevent the binding of genomic DNA to the Spin Filter. It will also reduce the viscosity of the lysate and prevent clogging.

10. Centrifuge the tubes at full speed ($\geq 16,000 \times g$) for 3 minutes.

What's happening: The lysate is thoroughly homogenized and all of the RNA is now free for binding to the Spin Filter.

11. Discard the DNA Shearing Spin Basket and add **350 μ l of Solution PX4** to the sample flow-through. Mix thoroughly with vortexing.

What's happening: Solution PX4 is 70% ethanol and will set up the final RNA binding conditions.

12. Transfer 600 μ l of sample onto the **Spin Filter (blue)**. Centrifuge at 10,000 $\times g$ for 1 minute to bind. Discard the flow through and put the **Spin Filter** back into the **2 ml Collection Tube**. Load the remaining lysate into the **Spin Filter** column and centrifuge at 10,000 $\times g$ for 1 minute to bind. Discard the flow-through and put the **Spin Filter** back into the **2 ml Collection Tube**.

What's happening: RNA is bound to the Spin Filter. The flow-through containing non RNA components is discarded. Multiple loads are required to process the entire sample volume through the filter.

13. Add **400 μ l of Solution PX5** to the **Spin Filter** column. Centrifuge at 10,000 $\times g$ for 1 minute. Discard the flow through and place the **Spin Filter** column into the same **2 ml Collection Tube** and centrifuge at 10,000 $\times g$ for an additional 2 minutes. This step will ensure the complete removal of Solution PX5 before the DNase I treatment.
Note: Incomplete removal of Solution PX5 may inhibit the DNase I activity.

What's happening: Solution PX5 is used to wash the Spin Filter in preparation for the on column DNase I digestion. Complete removal of Solution PX5 is required for efficient and complete DNase I digestion.

DNase I Treatment:

14. Transfer the **Spin Filter** to a new **2 ml Collection Tube** (provided). To the center of the Spin Filter, add **50 μ l of DNase I Solution** (a mixture of **45 μ l of Solution PX6** and **5 μ l of DNase I, see Important Notes Before Starting section**). Incubate at room temperature for 15 minutes.

Note: Do not centrifuge the Spin Filter before the addition of Solution PX5 in step 15 below.

What's happening: DNase I is mixed with high activity digestion buffer and is used to completely remove genomic DNA from the Spin Filter.

15. To the **Spin Filter** column add **400 μ l Solution PX5** and centrifuge at 10,000 $\times g$ for 1 minute. Discard the flow-through and place the **Spin Filter** back into the **2 ml Collection Tube**.



What's happening: Solution PX5 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 Filter.

Final Washes:

16. Add **500 µl of Solution PX7** to the **Spin Filter**. Centrifuge at 10,000 x g for 1 minute. Discard the flow-through and place the **Spin Filter** back into the **2 ml Collection Tube**.
17. Repeat the wash by adding another **500 µl of Solution PX7** to the **Spin Filter**. Centrifuge at 10,000 x g for 1 minute. Discard the flow-through and place the **Spin Filter** back into the **2 ml Collection Tube**. Centrifuge the **Spin Filter** for 2 minutes at 10,000 x g to completely dry the membrane.

What's happening: Solution PX7 is an ethanol based wash buffer used to remove residual salt and contaminants on the Spin Filter in preparation for the release and elution of the bound RNA. Complete removal of all traces of the wash solution is critical.

RNA Elution:

18. Transfer the **Spin Filter** column to a new **2 ml Collection Tube** (provided). Add **50 µl Solution PX8 (Sterile Water)** to the center of the Spin Filter membrane. Incubate 1 minute at room temperature then centrifuge the **Spin Filter** at 10,000 x g for 1 minute to elute.

NOTE: Placing Solution PX8 in the center of the small white membrane of the Spin Filter will ensure that the entire membrane is completely wetted. This will result in a more efficient and complete release of the RNA from the silica membrane of the Spin Filter.

What's happening: Solution PX8 is highly pure water used to elute the RNA from the silica membrane of the Spin Filter.

19. Repeat the elution step by adding another **50 µl of PX8** to the Spin Filter membrane. Centrifuge at 10,000 x g for 1 minute.
20. Remove the **Spin Filter** and close the cap of the **2 ml Collection Tube**. The RNA can be stored at -80°C until ready for use.

Thank you for choosing the BiOstic™ Stabilized Blood RNA Isolation Kit.

Hints and Troubleshooting Guide

DNA Shearing Column or Spin Filter Clogging

If the blood sample has a high blood cell count, the pellets may be larger than normal or appear very dark in color. The pellet can also be affected by diet and hydration of the individual before blood draw. If clogging of the DNA Shearing Column occurs:

- Make sure to vortex the sample 3-4 times during the 55°C digestion step. Make sure Proteinase K was added.
- After the first three minute centrifugation, if lysate is in the upper chamber of the spin basket, pipette the lysate up and down to loosen the sample in the carbide beads and help redistribute the sample. Centrifuge for another 5 minutes at full speed at the highest setting of your centrifuge (16,000- 20,000 x g).
- If lysate has still not completely passed through the silica carbide beads, set up a second DNA Shearing column and transfer the unfiltered lysate out of the top of the first column and load it onto the second column. Centrifuge at full speed for 3 minutes.
- After a second centrifugation step, use only the flow-through fraction for the remainder of the protocol.
- If samples contain a higher than normal WBC count, split the sample into two preps to obtain maximal RNA recovery.
Clogging of the Spin Filter does not occur once the sample has passed through the DNA Shearing Column. If you still experience clogging:
 - Centrifuge to bind the lysate to the Spin Filter for 2 minutes at 16,000 x g.
 - The sample may have an abnormally high blood cell count. Apply the remaining lysate to a second Spin Filter.

Low yields of RNA

Yields of total RNA will vary from person to person and is based on the health of each individual. Average yields will be between 4 and 12 µg. If yields of RNA are below 4 ug, the following may be the reason.

- Make sure the PAXgene™ Blood RNA Tube is inverted to mix several times immediately after collection and before storage. The RNA will not be stabilized if the sample is not well mixed.
- Samples must be incubated for 2 hours at room temperature either before or after freezing. The 2 hour time period begins once samples have fully reached room temperature. Do not thaw samples at 37°C to speed thawing.
- Make sure to use Solution PX1 provided in your kit for washing the cell pellet. Solution PX1 will ensure a complete isolation of the cells. Do not use water.
- Use RNase-Free plasticware for the procedure to avoid the introduction of RNases.
- Use the DNase I provided with the kit. It is certified RNase-Free.

RNA Appears Degraded on Agarose Gels or on the Agilent BioAnalyzer

Solution PX3 contains highly denaturing chaotropic salts to completely inactivate RNases. The use of Beta mercaptoethanol (βME) will destroy RNases and should be added fresh to Solution PX3. If RNA still appears degraded, the problem may be caused by the following:

- Prepare Solution PX3 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.
- 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**”.

Hints and Troubleshooting Guide cont.

RNA Appears Degraded on Agarose Gels or on the Agilent BioAnalyzer cont.

- 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 the RNA degradation.
- Agilent recommends a 2 minute denaturation of RNA at 70°C to ensure the removal of secondary structure. Always perform this step.
- Samples collected and stored for longer than 6 months may not yield high integrity RNA. The validated storage limits for the PAXgene™ Blood RNA Tube are: room temperature for up to 72 hours, up to 5 days at 4°C, or up to 6 months at -20°C or -80°C.

RNA Floats Out of Well When Loaded on a Gel

Residual Solution PX7 Wash Buffer may be in the final sample. To ensure complete drying of the membrane after Solution PX7, 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 PX7. (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 times at step 17 by another minute.

RNA has Low A_{260/280} Ratio

The ratio for pure RNA should be 1.9-2.1. A 260/280 reading below 1.6 may have significant protein contamination.

- Make sure that the Solution PX5 wash was performed before and 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 BioOstic™ Stabilized Blood RNA Isolation Kit is provided with high quality RNase-Free DNase I for on-column digestion. When used with the Solution PX6 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 of RNA in sterile water (Solution PX8). If this is too dilute for your purposes, you may concentrate the RNA by adding 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.



Hints and Troubleshooting Guide cont.

Storing RNA

RNA is eluted in RNase-Free water (Solution PX8) 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% (12.3M) Formaldehyde
880 ml DEPC treated water

5x RNA Loading Dye

16 μ l Saturated aqueous Bromophenol blue solution
80 μ l .5 M EDTA, pH 8.0
720 μ l 37% (12.3M) Formaldehyde
2 ml 100% Glycerol
3084 μ l 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% (12.3M) Formaldehyde and 2 μ l 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

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 μ l of 5x RNA loading dye for each 8 μ l 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



Other Quality Products Available from MO BIO Laboratories, Inc.

For more product and detailed information go to www.mobio.com/catalog-request to request a catalog.

| DNA Purification and Gel Extraction | Catalog No. | Quantity |
|--|------------------------------------|---|
| PowerClean® DNA Clean-Up Kit | 12877-50 | 50 preps |
| UltraClean® 15 DNA Purification Kit | 12100-300 | 300 preps |
| UltraClean® PCR Clean-Up Kit | 12500-50 12500-100 12500-250 | 50 preps 100 preps 250 preps |
| UltraClean®-htp 96 Well PCR Clean-Up Kit | 12596-4 12596-12 | 4 x 96 preps 12 x 96 preps |
| UltraClean® GelSpin® DNA Extraction Kit | 12400-50 12400-100 12400-250 | 50 preps 100 preps 250 preps |
| Plasmid DNA Isolation | Catalog No. | Quantity |
| UltraClean® 6 Minute Mini Plasmid Prep Kit | 12300-50 12300-100 12300-250 | 50 preps 100 preps 250 preps |
| UltraClean® Standard Mini Plasmid Prep Kit | 12301-50 12301-100 12301-250 | 50 preps 100 preps 250 preps |
| UltraClean®-htp 96 Well Plasmid Prep Kit | 12396-4 12396-12 | 4 x 96 preps 12 x 96 preps |
| UltraClean® Midi Plasmid Prep Kit | 12700-20 12700-50 | 20 preps 50 preps |
| UltraClean® Maxi Plasmid Prep Kit | 12600-10 12600-20 | 10 preps 20 preps |
| UltraClean® Endotoxin-Free Mini Plasmid Prep Kit | 12311-100 12311-250 | 100 preps 250 preps |
| UltraClean® Endotoxin-Free Midi Plasmid Prep Kit | 12711-10 | 10 preps |
| UltraClean® Endotoxin-Free Maxi Plasmid Prep Kit | 12611-10 | 10 preps |
| UltraClean® Endotoxin Removal Kit | 12615 | 1 kit |
| UltraClean® Endotoxin-Free Ethanol Precipitation Kit | 12616 | 1 kit |
| UltraClean® Endotoxin Removal Reagent | 12625-25 | 25 ml |
| Endotoxin-Free Sodium Chloride | 12626-15 | 15 ml |
| Endotoxin-Free Centrifuge Tubes | 12617-100 12618-50 12619-25 | 100 each/2 ml tubes 50 each/15 ml tubes 25 each/50 ml tubes |
| RNA Isolation | Catalog No. | Quantity |
| PowerWater® RNA Isolation Kit (No Filters) | 14700-50-NF | 50 preps |
| PowerLyzer™ UltraClean® Tissue & Cells RNA Isolation Kit | 15055-50 | 50 preps |
| PowerLyzer™ UltraClean® Plant RNA Isolation Kit | 13355-50 | 50 preps |
| PowerBiofilm™ RNA Isolation Kit | 25000-50 | 50 preps |
| LifeGuard™ Soil Stabilization Solution | 12868-100 12868-1000 | 100 ml 1 L |
| On-Spin Column DNase I Kit (RNase-Free) | 15100-50 | 50 preps |
| BiOstic® Stabilized Blood RNA Isolation Kit | 12231-50 12231-100 | 50 preps 100 preps |
| BiOstic® Blood Total RNA Isolation Kit | 12230-50 | 50 preps |

| RNA Isolation ... Continued | Catalog No. | Quantity |
|---|-----------------------|-------------------------------|
| RNA PowerSoil® DNA Elution Accessory Kit | 12867-25 | 25 preps |
| RNA PowerSoil® Total RNA Isolation Kit | 12866-25 | 25 preps |
| UltraClean® Microbial RNA Isolation Kit | 15800-50 15800-250 | 50 preps 250 preps |
| UltraClean® Tissue & Cells RNA Isolation Kit | 15000-50 15000-250 | 50 preps 250 preps |
| UltraClean® Plant RNA Isolation Kit | 13300-20 13300-50 | 20 preps 50 preps |
| Genomic DNA Isolation | Catalog No. | Quantity |
| PowerLyzer™ PowerSoil® DNA Isolation Kit | 12855-50 12855-100 | 50 preps 100 preps |
| PowerLyzer™ UltraClean® Microbial DNA Isolation Kit | 12255-50 | 50 preps |
| PowerBiofilm™ DNA Isolation Kit | 24000-50 | 50 preps |
| PowerFood™ Microbial DNA Isolation Kit | 21000-50 21000-100 | 50 preps 100 preps |
| BiOstic® Bacteremia DNA Isolation Kit | 12240-50 | 50 preps |
| BiOstic® FFPE Tissue DNA Isolation Kit | 12250-50 | 50 preps |
| BiOstic® Paraffin Removal Reagent | 12251-50 | 2 x 25 ml |
| PowerMax® Soil DNA Isolation Kit | 12988-10 | 10 preps |
| PowerSoil® DNA Isolation Kit | 12888-50 12888-100 | 50 preps 100 preps |
| PowerSoil®-htp 96 Well Soil DNA Isolation Kit | 12955-4 12955-12 | 4 x 96 preps 12 x 96 preps |
| UltraClean® Soil DNA Isolation Kit | 12800-50 12800-100 | 50 preps 100 preps |
| UltraClean®-htp 96 Well Soil DNA Isolation Kit | 12896-4 12896-12 | 4 x 96 preps 12 x 96 preps |
| UltraClean® Mega Soil DNA Isolation Kit | 12900-10 | 10 preps |
| PowerClean® DNA Clean-Up Kit | 12877-50 | 50 preps |
| UltraClean® Fecal DNA Isolation Kit | 12811-50 12811-100 | 50 preps 100 preps |
| PowerMicrobial® Midi DNA Isolation Kit | 12225-25 | 25 preps |
| PowerMicrobial® Maxi DNA Isolation Kit | 12226-25 | 25 preps |
| UltraClean® Microbial DNA Isolation Kit | 12224-50 12224-250 | 50 preps 250 preps |
| UltraClean®-htp 96 Well Microbial DNA Isolation Kit | 10196-4 10196-12 | 4 x 96 preps 12 x 96 preps |
| PowerPlant® DNA Isolation Kit | 13200-50 13200-100 | 50 preps 100 preps |
| UltraClean® Plant DNA Isolation Kit | 13000-50 13000-250 | 50 preps 250 preps |



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| Genomic DNA Isolation ...Continued | Catalog No. | Quantity |
|--|--------------------------------|--------------------------------|
| UltraClean®-htp 96 Well Plant DNA Isolation Kit | 13096-4 13096-12 | 4 x 96 preps 12 x 96 preps |
| UltraClean® Tissue & Cells DNA Isolation Kit | 12334-50 12334-250 | 50 preps 250 preps |
| UltraClean®-htp 96 Well Tissue DNA Isolation Kit | 12996-4 12996-12 | 4 x 96 preps 12 x 96 preps |
| UltraClean® Blood DNA Isolation Kit (Non-Spin) | 12000-100 | 100 preps |
| UltraClean® Blood DNA Isolation Kit (Processes 1,000 ml of Blood) | 12000-1000 | 1 kit |
| UltraClean® Blood DNA Isolation Kit Plus RNase (Processes 1,000 ml of Blood) | 12002-1000 | 1 kit |
| UltraClean® BloodSpin® DNA Isolation Kit | 12200-50 12200-250 | 50 preps 250 preps |
| UltraClean®-htp 96 Well BloodSpin® DNA Isolation Kit | 12296-4 12296-12 | 4 x 96 preps 12 x 96 preps |
| UltraClean® Forensic DNA Isolation Kit | 14000-10 14000-20 | 10 isolations 20 isolations |
| PowerWater® Sterivex DNA Isolation Kit | 14600-50-NF | 50 preps |
| PowerWater® DNA Isolation Kit (No Filters) | 14900-50-NF 14900-100-NF | 50 preps 100 preps |
| RapidWater™ DNA Isolation Kit (No Filters) | 14810-50-NF 14810-100-NF | 50 preps 100 preps |
| UltraClean® Water DNA Isolation Kit (No filters) | 14800-10-NF 14800-25-NF | 10 preps 25 preps |
| Microbiological Culture Media | Catalog No. | Quantity |
| TB DRY® Powder Growth Media | 12105-05 12105-1 12105-5 | 500 g 1 kg 5 kg |
| LB Broth Powder Growth Media, pH 7 | 12106-05 12106-1 12106-5 | 500 g 1 kg 5 kg |
| LB Agar Powder Growth Media, pH 7 | 12107-05 12107-1 12107-5 | 500 g 1 kg 5 kg |
| LB Broth (Lennox) Powder Growth Media, pH 7 | 12108-05 12108-1 12108-5 | 500 g 1 kg 5 kg |
| LB Agar (Lennox) Powder Growth Media, pH 7 | 12109-05 12109-1 12109-5 | 500 g 1 kg 5 kg |
| Soybean-Casein Digest Medium (TSB), USP | 12114-05 12114-1 12114-5 | 500 g 1 kg 5 kg |
| Soybean-Casein Digest Agar Medium (TSA), USP | 12115-05 12115-1 12115-5 | 500 g 1 kg 5 kg |
| Yeast Extract | 12110-05 12110-1 12110-5 | 500 g 1 kg 5 kg |
| Tryptone | 12111-05 12111-1 12111-5 | 500 g 1 kg 5 kg |
| Agar, Bacteriological Grade | 12112-05 12112-1 12112-5 | 500 g 1 kg 5 kg |

| Other Reagents and Lab Accessories | Catalog No. | Quantity |
|---|--|--|
| 20 bp DNA Ladder | 17020-40 | 40 µg |
| 100 bp DNA Ladder | 17100-40 | 40 µg |
| 1 kb DNA Ladder | 17200-100 | 100 µg |
| UltraClean® Agarose, Molecular Biology Grade | 15003-50 15003-100 15003-500 15003-1000 | 50 g 100 g 500 g 1 kg |
| UltraClean® MS-8 Agarose | 15515-50 15515-100 15515-500 | 50 g 100 g 500 g |
| UltraClean® Forensic Agarose | 15505-50 15505-100 15505-500 | 50 g 100 g 500 g |
| UltraClean® Low Melt Agarose | 15005-50 15005-100 15005-500 | 50 g 100 g 500 g |
| UltraClean® Low Melt Sieve Agarose | 15004-50 15004-100 15004-500 | 50 g 100 g 500 g |
| Ethidium Bromide Solution | 15006-1 15006-10 | 1 ml 10 ml |
| Ethidium Bromide Destaining Tea Bags | 15007-25 | 25 bags |
| Dye Dots™ Dry Gel Loading Dye with Bromophenol Blue | 15020-10 15020-20 | 10 plates 20 plates |
| Bromophenol Blue Gel Loading Buffer | 15008-1 15008-5 | 1 ml 5 x 1 ml |
| Bromophenol Blue/Xylene Cyanol Gel Loading Buffer | 15009-1 15009-5 | 1 ml 5 x 1 ml |
| TAE Buffer, 50X (Tris-acetate-EDTA) | 15001-500 15001-1000 | 500 ml 1 liter |
| TBE Buffer, 10X (Tris-borate-EDTA) | 15002-500 15002-1000 | 500 ml 1 liter |
| RNase-Free Gloves | 1555-XS 1555-S 1555-M 1555-L | bag of 100 bag of 100 bag of 100 bag of 100 |
| UltraClean® Lab Cleaner | 12095-250 12095-500 12095-1000 | 250 ml squeeze bottle 500 ml spray bottle 1 liter bottle |
| KAPA PROBE FAST qPCR Kits | 51220-100 51220-500 51220-1000 | 100 reactions 500 reactions 1000 reactions |
| KAPA SYBR® FAST Universal 2X qPCR Master Mix | 51230-100 51230-500 51230-1000 | 100 reactions 500 reactions 1000 reactions |
| KAPA2G Robust HotStart ReadyMix | 51240-100 51240-500 | 100 reactions 500 reactions |
| KAPA HiFi HotStart ReadyMix | 51250-100 51250-500 | 100 reactions 500 reactions |
| KAPA2G FAST HotStart DNA Polymerase with dNTPs | 51260-100 51260-250 51260-500 | 100 reactions 250 reactions 500 reactions |



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| Other Reagents and Lab Accessories... Continued | Catalog No. | Quantity | Instrumentation and Accessories... Continued | Catalog No. | Quantity |
|--|--|---|---|--|---|
| KAPA2G FAST HotStart ReadyMix | 51270-100 51270-500 | 100 reactions 500 reactions | Garnet Bead Tubes, 0.70 mm | 13123-50 | 50 x 2 ml tubes |
| KAPA Long Range HotStart DNA Polymerase with dNTPs | 51280-100 51280-250 51280-500 | 100 reactions 250 reactions 500 reactions | Garnet + ¼ Ceramic 15 ml Bead Tubes, 0.70 mm | 13134-50 | 50 tubes |
| KAPA Taq Polymerase ReadyMix | 51290-250 | 250 reactions | Garnet + ¼ Ceramic 50 ml Bead Tubes, 0.70 mm` | 13144-10 13144-50 13144-100 13144-500 | 10 tubes 50 tubes 100 tubes 500 tubes |
| DNase (RNase-Free) | 15600-5 15601-100 | 5 mg 2500 units | Glass 15 ml Bead Tubes, 0.1 mm | 13135-50 | 50 tubes |
| Proteinase K | 1223-100 1222-2 | 100 mg 2 ml (20 mg/ml) | Glass 50 ml Bead Tubes, 0.1 mm | 13145-10 13145-50 13145-100 13145-500 | 10 tubes 50 tubes 100 tubes 500 tubes |
| Ribonuclease A (25 mg/ml) | 1202-1 1202-5 | 1 ml 5 ml | Glass 15 ml Bead Tubes, 1.0 mm | 13136-50 | 50 tubes |
| PCR Water | 17000-1 17000-5 17000-10 17000-11 | 1 ml 5 x 1 ml 10 x 1 ml 10 ml bottle | Ceramic 15 ml Bead Tubes, 1.4 mm | 13137-50 | 50 tubes |
| Molecular Biology Grade Water | 17012-200 17012-5200 | 200 ml 5 x 200 ml | Ceramic 50 ml Bead Tubes, 1.4 mm | 13147-10 13147-50 | 10 tubes 50 tubes |
| DEPC Treated Water | 17011-200 17011-5200 | 200 ml 5 x 200 ml | Metal 50 ml Bead Tubes, 2.38 mm | 13149-10 13149-50 | 10 tubes 50 tubes |
| Endotoxin-Free Water | 17013-10 17013-50 17013-100 17013-500 | 10 ml 50 ml 100 ml 500 ml | Ceramic Beads, 1.4 mm | 13113-325 | 325 gm |
| UltraClean® 5N NaOH | 17300-30 | 30 ml | Ceramic Beads, 2.8 mm | 13114-325 | 325 gm |
| UltraClean® 0.1N NaOH | 17301-30 | 30 ml | Glass Beads, 0.5 mm | 13116-400 | 400 gm |
| UltraClean® 0.5M EDTA, pH 8.0 | 17310-100 | 100 m | Metal Beads, 2.38 mm | 13117-500 | 500 gm |
| UltraClean® TE-4 Buffer | 17320-1000 | 1L | Glass Beads, 0.1 mm | 13118-400 | 400 gm |
| UltraClean® TE Buffer | 17325-1000 | 1L | Carbide Beads, 0.25 mm | 13121-500 | 500 gm |
| UltraClean® 1X PBS, pH 7.4 | 17330-500 | 500 ml | Garnet Beads, 0.15 mm | 13122-500 | 500 gm |
| UltraClean® 2M Tris, pH 8.0 | 17370-250 | 250 ml | Garnet Beads, 0.70 mm | 13123-05 | 500 gm |
| UltraClean® 0.1M Tris, pH 7.0 | 17371-1000 | 1L | PowerMix 15 ml Bead Tubes | 13138-50 | 50 tubes |
| Instrumentation and Accessories | Catalog No. | Quantity | PowerMix 50 ml Bead Tubes | 13148-10 13148-50 | 10 tubes 50 tubes |
| PowerLyzer™ 24 Bench Top Bead-Based Homogenizer (110/220V) | 13155 | 1 unit | 2 ml Collection Tubes | 1200-100-T 1200-150-T 1200-250-T | 100 tubes 150 tubes 250 tubes |
| PowerLyzer™ Tube Holder | 13156 | 1 unit | 2 ml Screw Cap Tubes | 12800-200-E | 200 tubes & caps |
| PowerLyzer™ Tube Holder Stand | 13157 | 1 unit | 15 ml Collection Tubes | 12700-T | 25 tubes |
| PowerVac™ Mini System | 11992 | 1 unit + 20 adapters | 50 ml Centrifuge Tubes | 12600-T | 25 tubes |
| PowerVac™ Manifold | 11991 | 1 unit | Spin Filters (in 1.9 ml tubes) | 1200-50-SF 1200-100-SF 1200-250-SF | 50 filters 100 filters 250 filters |
| PowerVac™ Mini Spin Filter Adapters | 11992-10 11992-20 | 10 adapters 20 adapters | Endotoxin-Free Centrifuge Tubes | 12617-100 12618-50 12619-25 | 100 each/2 ml tubes 50 each/15 ml tubes 25 each/50 ml tubes |
| Ceramic Bead Tubes, 1.4 mm | 13113-50 | 50 bead tubes | 15 ml Midi Spin Filters | 12700-SF | 25 spin filters |
| Ceramic Bead Tubes, 2.8 mm | 13114-50 | 50 bead tubes | | | |
| Glass Bead Tubes, 0.5 mm | 13116-50 | 50 bead tubes | | | |
| Glass Bead Tubes, 0.1 mm | 13118-50 | 50 bead tubes | | | |
| Metal Bead Tubes, 2.38 mm | 13117-50 | 50 bead tubes | | | |
| 2.0 ml Tough Tubes with Cap | 13119-500 13119-1000 | 500 1000 | | | |
| Carbide Bead Tubes, 0.25 mm | 13121-50 | 50 x 0.5 ml tubes | | | |
| Garnet Bead Tubes, 0.15 mm | 13122-50 | 50 x 0.5 ml tubes | | | |

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



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| Instrumentation and Accessories... <i>Continued</i> | Catalog No. | Quantity |
|---|----------------------------------|--|
| Vortex-Genie® 2 Vortex (120V) | 13111-V | 1 unit |
| Vortex-Genie® 2 Vortex (220V) | 13111-V-220 | 1 unit |
| Vortex Adapter, holds 12 (1.5-2.0 ml) tubes | 13000-V1 | 1 unit |
| Vortex Adapter, holds 6 (5 ml) tubes | 13000-V1-5 | 1 unit |
| Vortex Adapter, holds 4 (15 ml) tubes | 13000-V1-15 | 1 unit |
| Vortex Adapter, holds 2 (50 ml) tubes | 13000-V1-50 | 1 unit |
| Vortex Adapter, holds 24 (1.5-2.0 ml) tubes | 13000-V1-24 | 1 unit |
| Anti-Static Funnels, Micro | 23301-96 | Pack of 96 |
| Anti-Static Funnels, Small | 23302-50 | Pack of 50 |
| Anti-Static Funnels, Medium | 23303-50 | Pack of 50 |
| Anti-Static Funnels, Large | 23304-20 | Pack of 20 |
| Mini Horizontal Gel System | 16001 | 1 each |
| Mini Horizontal Gel Caster, 3 place | 16003 | 1 each |
| Mini Horizontal Gel Tray | 16004 | 1 each |
| Polycarbonate Single-sided Comb | 16005 16006 16007 16008 | 1 mm x 3 well 1 mm x 8 well 1 mm x 10 well 1 mm x 12 well |
| Polycarbonate Dual-sided Comb | 16013 16014 16015 16016 | 1 mm x 8 well/16 well 1 mm x 10 well/14 well 2 mm x 8 well/16 well 2 mm x 10 well/14 well |
| Teflon Single-sided Comb | 16009 16010 16011 16012 | 1 mm x 3 well 1 mm x 8 well 1 mm x 10 well 1 mm x 12 well |
| Teflon Dual-sided Comb | 16017 16018 16019 16020 | 1 mm x 8 well/16 well 1 mm x 10 well/14 well 2 mm x 8 well/16 well 2 mm x 10 well/14 well |
| Power Supply w/Timer, (120V) | 16023 | 1 unit |
| 96 Well Plate Shaker (120V) | 11996 | 1 unit |
| 96 Well Plate Shaker (220V) | 11996-220 | 1 unit |
| Plate Adapter Set | 11999 | 1 set |
| Vacuum Pump (120V) | 11998 | 1 unit |
| Vacuum Pump (220V) | 11998-220 | 1 unit |