



Instruction Manual

FastRNA™ Pro Blue Kit

Rapid Isolation of Total RNA from Gram Positive and Gram Negative Bacteria using the FastPrep® Instrument

- One Call
- One Source
- A World of Biotechnology Reagents

Size:
50 preps

Storage:
Refrigerated or Ambient temperature
(4° C or 15 - 30° C)

Cat. No. 116025050

Protocol Revision # 116025050-201411

DO NOT expose RNApro™ Solution to light for extended periods of time. Store in the original bottle in the closed kit box.

Note: An empty space in the box insert has been provided for convenient storage and access to the RNApro™ Solution when it has been removed from the safety shipping container.

MSDS now available online

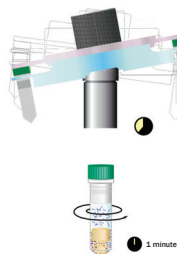
www.mpbio.com/sampleprep



FastPrep® Family Workflow

Select the Best Homogenization and Extraction Solution for your Application.

Homogenization



Instruments



FastPrep-24™ 5G



FastPrep-96™



Super FastPrep-1™

Adapters



Metal



Cryogenic



High throughput



Large Sample volume

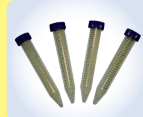
Lysing Matrix



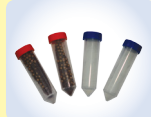
2mL



4,5mL

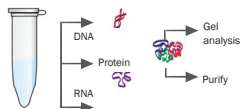


15mL



50mL

Extraction



DNA, RNA & Protein Isolation Kits



DNA



RNA



Protein

FastRNA™ Pro Blue Kit

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and Gram Negative Bacteria using
the FastPrep® Instrument

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1. Introduction to the FastRNA™ Pro Blue Kit and the FastPrep® Instrument

The FastRNA™ Pro Blue Kit is a single reagent extraction method designed to quickly and efficiently isolate total cellular RNA from gram positive and gram negative bacteria. The RNApro™ Solution included in the kit is designed to efficiently inactivate cellular RNases during cell lysis to prevent RNA degradation. During use the RNApro™ Solution is mixed with the bacterial sample in a tube containing a specifically selected lysing matrix. The tube is then processed in the FastPrep® Instrument for 40 seconds to release the total cellular RNA, DNA and proteins. Following the FastPrep® homogenization the RNA is purified and isolated by chloroform extraction and ethanol precipitation. The purified RNA is ready for downstream applications, including RT-PCR and northern analysis. The average RNA yield from 10¹⁰ bacteria is greater than 50 µg.

The FastPrep® Instrument is a high-speed, benchtop device that uses a proprietary vertical angular motion 1) to produce sample homogenization by simultaneous matrix impaction from multiple directions. The FastPrep® Instrument provides an extremely quick and highly reproducible homogenization that surpasses traditional lysis methods using enzyme digestion, sonication, blending, douncing and vortexing. When used with FastPrep® kits the FastPrep® Instrument permits the release and purification of intact DNA, RNA and proteins from virtually any source, including bacteria, yeast and fungi, spores, plant seeds and leaves, animal tissue, organs and blood, etc.

2. Kit Components and User Supplied Materials

2.1 FastRNA™ Pro Blue Kit Components

<u>Product Description</u>	<u>Qty.</u>	
RNApro™ Solution	2 x 27.5 mL bottles	Cat. No. 116055050
DEPC-H ₂ O	1 x 15 mL bottle	Cat. No. 111007201
Lysing Matrix B	50 x 2 mL tubes	Cat. No. 116911050
Short protocol	1 each	
User manual	1 each	
MSDS	1 each	
Certificate of Analysis	1 each	

2.2 User Supplied Materials

FastPrep® Instrument (See section)
Microcentrifuge
Pipettmen
RNase Erase® (Cat # 112440204), recommended
Chloroform
100% ethanol
75% ethanol
1.5 or 2.0 mL RNase-free microcentrifuge tubes
Agarose
Gel loading dye and RNA size marker

3. Important Considerations before Use

The presence or introduction of RNase during the procedure may result in sample degradation. It is strongly recommended that the user minimize the potential for RNase contamination by using gloves throughout the procedure, using DEPC-H₂O and by treating pipettmen, work area, gel box and gel comb with RNase Erase®. Additional RNA handling methods and precautions may be found in references 2 and 3.

The volume after the addition of RNApro™ Solution to the sample has been calculated to maintain a sufficient air space in the sample tube during FastPrep® Instrument processing. Sample loss or tube failure may result from overfilling the matrix tube. The matrix tube caps must be secure, but not over-tightened, to prevent sample leakage. If the sample is too large for processing in a single tube, divide the sample and process using multiple tubes.

Confirm the sample tubes spin freely and will not scrape the microcentrifuge wall during centrifugation.

The use of other manufactured tubes in the FastPrep® Instrument is not recommended and may result in sample loss or FastPrep® Instrument failure.

Add the RNApro™ Solution to the sample as soon as possible to initiate RNase inhibition.

Samples, both FastPrep® Instrument homogenized and non-homogenized, are stable in RNApro™ Solution overnight at room temperature or 4°C.

Bacterial strain variability may result in unwanted protein and mucopolysaccharide carryover into the aqueous solution following chloroform extraction. While this may not compromise downstream applications the user may adapt the protocol to include an additional chloroform (isoamyl alcohol may be included with the chloroform [CHCl₃:IAA, 24:1, v:v]) extraction after Step 10 (Quick Protocol for Experienced Users) or in Step 12 (Detailed Procedure) to reduce the potential carryover.

A single 40 second run at a speed setting of 6.0 in the FastPrep® Instrument is sufficient to lyse a bacterial sample. If the user determines that additional processing steps in the FastPrep® Instrument are required to homogenize a sample it is recommended that the sample be incubated on ice in the sample tube for at least 2 minutes between successive FastPrep® Instrument homogenizations to prevent sample heating and possible RNA degradation.

The FastRNA™ Pro Blue Kit is designed to selectively purify total cellular RNA from DNA and protein. Experiments have indicated the RNA is sufficiently pure for use in RT-PCR and northern analysis, however, it is recommended the user incorporate DNase I treatment of the RNA prior to use in applications where absolute control of DNA contamination is essential. Use DNase I at the concentration recommended by the manufacturer and incubate at 37°C for 30 minutes. The DNase I is inactivated by incubation at 75°C for 5 minutes or by addition of EDTA to 25 mM followed by phenol/chloroform extraction and precipitation (2, 3).

4. Safety Precautions

The RNApro™ Solution contains components that when in contact with human tissue or during inhalation may cause irritation or burning. Wear personal protective equipment to prevent skin contact (e.g., gloves, lab coat, and eye protection) and prevent inhalation of reagent vapors and consumption of liquid during use. Consult the enclosed Material Safety Data Sheet for additional details.

5. Protocol

1. Dilute 1 mL of an overnight bacterial culture into 14 mL of fresh media in a sterile 50 mL tube or 250 mL flask.
2. Incubate for ~4–6 hours at 37°C with shaking at ~150–200 rpm to reach an OD600 = 0.9–1.0 (Note: 1.0 OD600 for *Escherichia coli* is ~1 X 10⁹ cells per milliliter).
3. Remove 10 mL of the culture to a 15 mL conical tube and pellet the cells by centrifugation at 2,800 rpm (x 1,500 g) for 15 minutes at 4°C (e.g., Beckman Model TJ-6 Centrifuge, I-92 Swinging Bucket Rotor) for 10 minutes.
4. Decant the supernatant and add 1 mL of RNApro™ Solution to the tube. Completely resuspend the cells by pipetting or vortexing.
5. Transfer 1 mL of the resuspended cells to a blue-cap tube containing Lysing Matrix B provided in the kit. Securely close the cap to prevent leakage in the next step.
NOTE: The calculated volumes will provide adequate airspace in the matrix tube to prevent sample leakage and/or tube failure. DO NOT overfill the matrix tube. To process a greater number of cells use a second matrix tube.
6. Process the sample tube in the FastPrep® Instrument for 40 seconds at a setting of 6.0.
7. Remove the sample tube and centrifuge at a minimum of 12,000 x g for 5 minutes at 4°C or room temperature.
8. Transfer the liquid (~750 µL) to a new microcentrifuge tube. Avoid transferring the debris pellet and lysing matrix.
9. Incubate the transferred sample 5 minutes at room temperature to increase RNA yield.
10. Add 300 µL of chloroform (NO isoamyl alcohol). Vortex 10 seconds.
11. Incubate 5 minutes at room temperature to permit nucleoprotein dissociation and increase RNA purity.
12. Centrifuge the tubes at a minimum of 12,000 x g for 5 minutes at 4°C.

Samples containing large amounts of cellular mucopolysaccharides can be re-extracted with chloroform (isoamyl alcohol may be included with the chloroform [CHCl₃:IAA, 24:1, v:v]) to increase RNA purity. Alternatively, a lithium chloride precipitation may be used (see the Troubleshooting section and references 3, 4).

13. Transfer the upper phase to a new microcentrifuge tube without disturbing the interphase. If a portion of the interphase is transferred, repeat the centrifugation with the upper phase, and transfer the new upper phase to a clean microcentrifuge tube.

14. Add 500 µL of cold absolute ethanol to the sample, invert 5X to mix and store at -20°C for at least 30 minutes.

15. Centrifuge at a minimum of 12,000 x g for 15 minutes at 4°C and remove the supernatant. The RNA will appear as a white pellet in the tube. If the pellet is floating the sample may be recentrifuged to place the pellet at the tube bottom.

16. Wash the pellet with 500 µL of cold 75% ethanol (made with DEPC-H₂O).

17. Remove the ethanol, air dry 5 minutes at room temperature (DO NOT completely dry the RNA) and resuspend the RNA in 100 µL of DEPC-H₂O for short-term storage. RNA is generally stable for up to a year at -80°C. For longer term storage RNA samples may be stored at -20°C as ethanol precipitates. When stored as an ethanol precipitate, the RNA must be precipitated and resuspended in aqueous solution prior to use. NOTE: RNA does not evenly distribute in ethanol and can lead to inconsistent RNA amounts between samples when equal volumes are pipetted.

Vortex the RNA:ethanol solution to disperse the RNA prior to removing the sample. In situations where precise amounts of RNA are required it is best to precipitate the total amount (or excess) of RNA required, resuspend the RNA in DEPC-H₂O and measure the concentration by OD260 before proceeding.

18. Incubate 5 minutes at room temperature to facilitate RNA resuspension.

19. Determine the RNA concentration:

- a. Dilute 5 µL of the purified RNA into 495 µL of DEPC-H₂O
- b. Read the OD260 using DEPC-H₂O as a blank



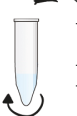
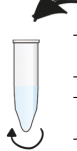


- c. Calculate the sample μg RNA per mL using the formula:
 $(\text{OD}_{260})(40 \mu\text{g}/\text{mL}/\text{per OD})(100 [\text{dilution factor}]) = \mu\text{g RNA per mL}$

Spectrophotometer accuracy is greatest between ~ 0.2 and ~ 0.8 . If the OD reading is below the range, add more RNA sample (e.g., $20 \mu\text{L RNA} + 480 \mu\text{L DEPC-H}_2\text{O}$) or concentrate the RNA by precipitation and resuspension into a smaller volume. If the OD reading is above the recommended spectrophotometer range, use less RNA for the OD determination.

20. Aliquot and store the RNA solution at -70°C .

21. The RNA integrity can be determined by analyzing a portion of the RNA sample using gel electrophoresis. Add $1 \mu\text{g RNA}$ in $9 \mu\text{L DEPC-H}_2\text{O}$, heat to 65°C for 5 minutes, add gel loading buffer (see Related Products) and load the sample on a 1.2% agarose gel containing 2.2M formaldehyde in MOPS buffer. The sample is run at ~ 80 volts for 30 minutes (2, 3). Ethidium bromide may be added to the denatured RNA sample at a final concentration of $10 \mu\text{g}$ per milliliter prior to gel loading or the gel may be ethidium bromide stained and destained following electrophoresis and visualized under UV light. The quality of the RNA is determined by the appearance of the large, 23S, and small, 16S ribosomal RNAs as sharp, distinct bands of 2.9 and 1.5 kb. Heterogeneous-sized messenger RNA may appear as diffuse ethidium staining between and below the ribosomal bands. Small RNA species such as tRNA and 5S RNA may be present in varying amounts at the dye front.

FastRNA™ Pro Blue KIT

<h2>1. Prepare the sample</h2>	 <p> 15 mins 1 500 g 4 °C </p> <ul style="list-style-type: none"> - Dilute 1 ml of an overnight bacterial culture into 14 ml of fresh media. - Remove 10 ml of the culture to a 15 ml canonical tube - Centrifuge to pellet cells - Decant the supernatant and add 1 ml of RNAPro Solution to the tube. - Resuspend the cells by pipetting <p>15 ml Tube</p>
<h2>2. Homogenize in the FastPrep instrument</h2>	 <p> 40 sec. 5 mins 12 000 g 4 °C </p> <ul style="list-style-type: none"> - Transfer 1 ml of the bacterial solution mixture to a Lysing Matrix B tube. - Load tube in FastPrep instrument. Process 40s at a speed setting of 6.0m/s <p>Centrifuge to pellet debris.</p>
<h2>3. Extract RNA</h2>	 <p> 2 mins 12 000 4 °C </p> <ul style="list-style-type: none"> - Transfer supernatant to a clean 2ml microcentrifuge tube. - Incubate 5 mins at Room Temperature (RT) - Add 300µL of chloroform, vortex 10 sec and incubate 5 mins at RT & centrifuge. <p>2 ml Tube</p>
<h2>4. Precipitate RNA</h2>	 <p> 15 mins 12 000 g 4 °C </p> <ul style="list-style-type: none"> - Transfer the upper phase to a new microcentrifuge tube. - Add 500µL of cold absolute ethanol - Invert 5x to mix and store at -20 °C for 30 mins at least. - Centrifuge & remove the supernatant <p>2 ml Tube</p>
<h2>5. Wash the precipitated RNA</h2>	 <ul style="list-style-type: none"> - Add 500 µL of cold 75% ethanol (made with DEPC-H₂O) - Remove the ethanol <p>2 ml Tube</p>
<h2>6. Dry and Resuspend the RNA</h2>	 <ul style="list-style-type: none"> - Air dry 5 mins at Room Temperature (<i>do not completely dry the RNA</i>). - Resuspend the RNA in 100µL of DEPC-H₂O. - Incubate 5 mins at Room Temperature. <p>- RNA is ready to use</p>

6. Table of Typical FastPrep® Settings

Sample Name	Sample type	Quantity	Lysing Matrix	FastPrep speed	FastPrep Time
Bacteria					
<i>Listeria monocytogenes</i>	Cells	10 ⁹ cells	B	6.0	3x30 sec
<i>Streptococcus pyogenes</i>	Cells	10 ⁹ cells	B	6.0	20 sec
<i>Streptococcus mutants</i>	Cells	10 ⁹ cells	B	6.0	30 sec
<i>Streptococcus aureus</i>	Cells	10 ⁸ cells	B	6.0	2x40 sec
<i>Photorhabdus luminescens</i>	Cells	10 ⁹ cells	B	6.0	2x30 sec
<i>Escherischia coli</i>	Cells	10 ⁸ cells	B	6.0	30 sec
<i>Mycobacterium tuberculosis</i>	Cells	10 ⁸ cells	B	6.0	2x45 sec
<i>Lactococcus lactis</i>	Cells	10 ⁸ cells	B	6.0	3x30 sec
Yeast & Fungi					
<i>Saccharomyces cerevisiae</i>	Cells	2x10 ⁸ cells	C	6.0	40 sec
<i>Schizosaccharomyces pombe</i>	Cells	10 ⁸ cells	C	5.0	4x15 sec
<i>Candida albicans</i>	Cells	10 ⁸ cells	C	6.0	2x30 sec
<i>Cryptococcus neoformans</i>	Cells	10 ⁸ cells	C	6.0	4x30 sec
<i>Aspergillus fumigatus</i>	Cells	10 ⁸ cells	C	6.0	2x30 sec
<i>Fusarium solani</i>	Cells	10 ⁸ cells	C	6.0	2x30 sec

7. Troubleshooting

7.1 Degraded RNA or Lower than Expected RNA Yields

RNA purified using the FastRNA™ Pro Blue Kit and analyzed by denaturing or non-denaturing agarose gel electrophoresis will appear as 2 distinct ribosomal RNA (rRNA) bands of approximately equal fluorescent intensity using ethidium bromide staining. The rRNA bands will appear in the area between 2000 and 1000 nucleotides.

Messenger RNA (mRNA), which typically represents approximately less than 1% of the total cellular RNA and is heterogeneous length, will not be visible as distinct bands. rRNA is used as a marker to assess sample RNA degradation. Degraded RNA or mRNA may appear as unequal fluorescent intensity between bands, a single band may be completely lacking or a heterogeneous fluorescent smear may appear below the rRNA bands or throughout the gel lane.

Recommended precautions include cleaning all instruments and work area with RNase Erase® (Catalog # 112440204) prior to use. Use disposable sterile plastic containers when possible. Glassware should be thoroughly cleaned, rinsed with DEPC-H₂O and baked at 250°C for 4 hours to remove RNase. Sterile, plugged micropipettes are recommended (see 2, 3 for additional suggestions).

Certain bacterial strains may contain elevated RNase levels. Reduce the exposure time to RNase by adding RNapro™ Solution to each sample as soon as possible following sample harvest. Process fewer samples to shorten the time before complete cellular lysis and exposure to the RNase inactivating activity of RNapro™ Solution.

Bacteria in log phase growth with maximal aeration and nutrients provide the highest yield and integrity RNA. Bacterial cells in stationary phase, growing in oxygen or nutrient limiting conditions, stored for extended duration at room temperature or refrigerated for extended periods will contribute to reduced RNA yield and integrity.

RNapro™ Solution can permeate samples and will protect bacterial RNA from degradation for at least 24 hours before it is processed in the FastPrep® Instrument.

Artifactual RNA degradation may occasionally occur during gel electrophoresis due to a gel that was not RNase free, running the gel at too high voltage or from using depleted running buffer. Rerun the samples with a known intact RNA sample using freshly prepared reagents.

RNA degradation may occur due to RNase contamination introduced into the DEPC-H₂O following use. If contamination is suspected, prepare fresh DEPC-H₂O in an RNase free container (2, 3). RNapro™ Solution contains RNase inactivating components and will not support active RNase contamination.

7.2 No Pellet after Ethanol Precipitation

The purified RNA may not appear as a pellet but may instead adhere to the side of the tube. The RNA may not be visible and it MAY APPEAR THAT RNA HAS NOT BEEN PURIFIED. COMPLETE THE RNA PURIFICATION per the instructions provided and confirm the RNA concentration by OD₂₆₀ and integrity by gel electrophoresis. RNA adhering to the tube wall will not affect its purity, size or use in subsequent applications.

The RNA pellet may not be firmly attached to the side of the tube and may be observed floating in the solution or at the solution surface. Recentrifuge the sample in the same tube and exercise caution to not lose the pellet when removing the supernatant. Confirm enough sample was used to isolate RNA (1.0 OD for *Escherichia coli* ~1x10⁹ cells).

7.3 Genomic DNA Contamination

Genomic DNA contamination will appear as a high molecular weight smear on a denaturing gel or as ethidium bromide stained material in the gel loading well. In the event genomic DNA contamination occurs, treat sample with DNase according to the manufacturer's instructions.

7.4 Mucopolysaccharide / Carbohydrate Contamination

Samples containing large amounts of cellular mucopolysaccharides can be re-extracted after the initial chloroform extraction with a second chloroform extraction. Isoamyl alcohol may be included with the chloroform [CHCl₃:IAA, 24:1, v:v] to increase RNA purity. Refer also to Lithium Chloride Precipitation in the Troubleshooting section.

7.5 Lithium Chloride Precipitation

Lithium chloride (LiCl) may be used to precipitate RNA while excluding carbohydrate, DNA and proteins, including transcription inhibitors. Lithium chloride has historically been used to precipitate RNA greater than ~300 nucleotides from tRNA and 5S RNA. Lithium chloride precipitation may be incorporated into the FastRNA™ Pro Blue Kit procedure: Following ethanol precipitation of the RNA and resuspension in 100 µL

DEPC-H₂O, add lithium chloride to a final concentration of 2–3 M (e.g., 0.2 volumes [20 µL] RNase free 8 M lithium chloride). Add 2.5 volumes RNase free absolute ethanol (250 µL). Mix the solution and store on ice at least 2 hours. Centrifuge for 15 minutes at a minimum of 12,000 rpm at 4°C. Remove the supernatant and wash the pellet with 75% cold RNase free ethanol. The ethanol wash step is critical to prevent LiCl inhibition of cell-free translation and in vitro transcription. Air dry and resuspend the RNA in 100 µL DEPC-H₂O.

8. Related Products

Instruments	Voltage	Cat. No.
FastPrep-24™ 5G Instrument	100-230V	116005500
FastPrep-96™ Instrument	100-230V	116010500
Super FastPrep-1™ Instrument	100-230V	116011500
MPure-12™ Instrument	100-230V	117002200
Kits	Size	Cat. No.
FastDNA™ Kit	100 preps	116540400
FastDNA™ SPIN Kit	100 preps	116540600
FastDNA™ SPIN Kit for Soil	50 preps	116560200
FastDNA™ SPIN Kit for Feces	50 preps	116570200
FastRNA™ Pro Soil-Direct Kit	50 preps	116070050
FastRNA™ Pro Soil-Indirect Kit	50 Preps	116075050
FastRNA™ Pro Red Kit (Yeast & Fungus)	50 preps	116035050
FastRNA™ Pro Green Kit (Plant & Animal)	50 preps	116045050
FastRNA™ Pro Blue Kit (Bacteria)	50 preps	116025050
FastPROTEIN™ Blue Matrix	50 preps	116550400
FastPROTEIN™ Red Matrix	50 preps	116550600

9. References

1. U.S. Patent 5,567,050. Zbloninsky et.al, Apparatus and method for rapidly oscillating specimen vessels.
2. Molecular Cloning, Sambrook and Russell. Cold Spring Harbor Laboratory Press, 3rd Edition, 2001.
3. Current Protocols in Molecular Biology, John Wiley & Sons, Inc., 2002, www.currentprotocols.com.

10. Product Use Limitation & Warranty

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NOTE:

NOTE:

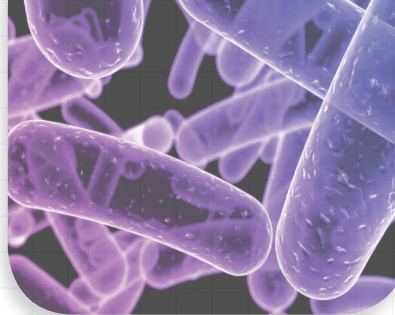
NOTE:

Instruction Manual

FastRNA™ Pro Blue Kit

Protocol Revision #116025050-120CT

Cat. No. and Size: 116025050, 50 preps



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