USER GUIDE

ambion®
by life technologies™

MagMAX™-96 Blood RNA Isolation Kit

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MagMAX™-96 Blood RNA Isolation Kit

IMPORTANT! Before using this kit, read and understand the information in the "Safety" appendix in this document.

Product description and procedure overview

The MagMAX™-96 Blood RNA Isolation Kit is designed for rapid, high throughput isolation of total and viral RNA from mammalian whole blood and milk in 96-well plates. 96 samples can be processed at once with the MagMAX™-96 Blood RNA Isolation Kit, however, it can also be used to efficiently isolate RNA from fewer than 96 samples.

The procedure (see Figure 1 on page 6) is fast (<1 hr), simple, and well suited for automation. First, 50 µL samples are subjected to vigorous denaturing lysis conditions for efficient cellular lysis, rapid release of total and viral RNA, inactivation of nucleases, and degradation of proteins. Magnetic beads with a nucleic acid binding surface are then added to the lysate. The beads and bound nucleic acids are then magnetically captured and washed to remove proteins and RT-PCR inhibitors. Next, the nucleic acid is treated with DNase, and purified from the reaction mixture using a second magnetic bead capture and washing procedure. Finally, RNA is eluted in 30 µL of low salt buffer. The recovered viral RNA or total RNA can be used directly for quantitative reverse transcriptase PCR (qRT-PCR) for veterinary molecular diagnostics or gene expression profiling. The MagMAX™-96 Blood RNA Isolation Kit is optimized for use either manually with multichannel pipettors, or with robotic liquid handlers.

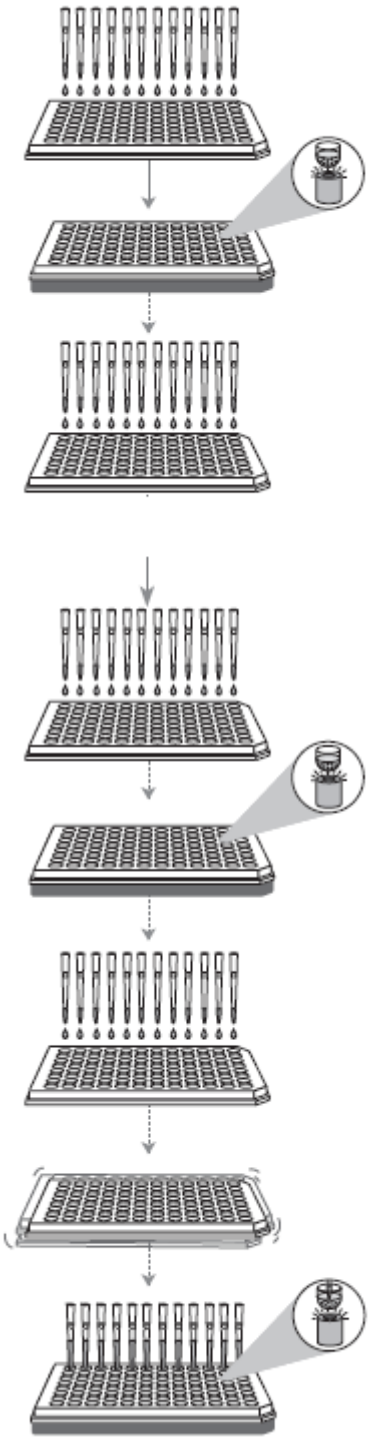
Whole blood is an ideal clinical sample source

Whole blood is an attractive sample source for assays involving nucleic acids because it is readily available, easily accessible, and rich in genetic information. Many protocols for RNA isolation from blood require sample preprocessing steps that can lead to sample mix-up and/or cross contamination. The MagMAX™-96 Blood procedure eliminates preprocessing by using whole blood directly for total RNA and viral RNA isolation.

- Total RNA from human whole blood

  Figure on page 7 illustrates that approximately 300–500 ng of intact RNA, with 28S/18S ribosomal RNA (rRNA) ratios 0.8–1, can consistently be purified from 50 µL of human whole blood. The recovered RNA is devoid of PCR inhibitors, such as proteins and heme, commonly found in RNA isolated from blood (data not shown).

Figure 1 Procedure overview



Sample lysis and initial nucleic acid purification

1.Add 50 µL sample to 130 µL Lysis/Binding Solution and mix by pipetting up and down at least five times. (page 11)

2.Add 20 µL of Bead Mix and shake for 5 min (page 12)

3.Magnetically capture the RNA Binding Beads and carefully discard the supernatant (page 12)

4.Wash with 150 µL Wash Solution 1 (page 12)

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TURBO DNase™ enzyme treatment and final clean-up

1.Prepare Diluted TURBO™ DNase (page 13)

2.Add 50 µL of Diluted TURBO DNase solution and shake for 5 min (page 13)

3.Add 130 µL Lysis/Binding Solution and shake for 3 min to rebind the RNA (page 13)

4.Magnetically capture the RNA Binding Beads and carefully discard the supernatant (page 13)

5.Wash the RNA Binding Beads twice with 150 µL Wash Solution 2 (page 13)

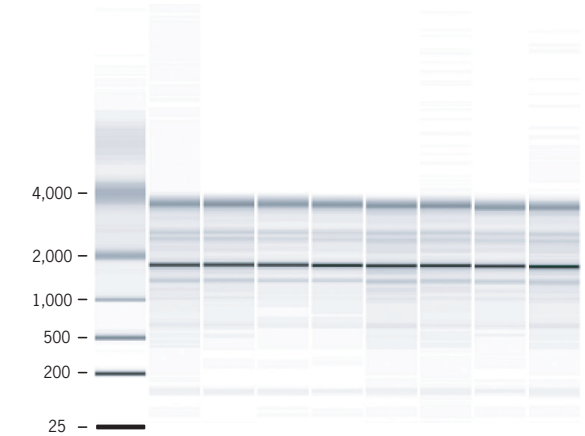
6.Dry the beads by shaking for 2 min (page 13)

7.Elute the RNA in 30–50 µL Elution Buffer (page 13)

- Total RNA from whole blood from other species

  Bovine, porcine, and ovine whole blood samples are commonly used for viral molecular diagnostics. Figure 3 shows total RNA yield from bovine, porcine, and ovine whole blood samples stored in sodium EDTA; RNA was also successfully isolated from whole blood stored in potassium EDTA, heparin, and sodium citrate (data not shown).

Figure 2 Consistent Yield, Purity, and Integrity of RNA Isolated from Human Whole Blood with the MagMAX™-96 Blood RNA Isolation Kit. Total RNA was isolated from 50 µL fresh human blood (single donor) in 8 replicates using the MagMAX™-96 Blood RNA Isolation Kit. Spectrophotometer readings were obtained by reading 2 µL of the purified RNA on a NanoDrop® ND-1000. The ratio of 28S to 18S rRNA was obtained by analyzing 3 µL of the RNA (~50 ng) on an RNA LabChip® Kit with an Agilent® 2100 Bioanalyzer™ instrument.



									Avg	Stdev
Yield (µg)	0.52	0.53	0.52	0.56	0.60	0.55	0.56	0.63	0.56	0.04
A_{260}/A_{280}	1.83	2.02	1.83	1.82	1.77	1.83	1.84	1.83	1.85	0.07
28S/18S rRNA Ratio	1.01	0.97	1.02	1.05	0.91	0.99	0.96	0.84	0.97	0.07

Figure 3 Consistent Total RNA Yield from Bovine, Porcine, and Ovine Whole Blood. Total RNA was isolated from 50 µL of fresh blood from the indicated species (single donor) in 8 replicates using the MagMAX™-96 Blood RNA Isolation Kit. Yield was determined by reading 2 µL of the purified RNA on a NanoDrop® ND-1000 spectrophotometer.

Species	Average yield	Standard deviation
Bovine	200 ng	50 ng
Porcine	940 ng	80 ng
Ovine	130 ng	40 ng

The MagMAX™-96 Blood procedure can be performed manually or by robotic liquid handling systems

The MagMAX™-96 Blood RNA Isolation Kit was designed to accommodate manual processing and robotic liquid handling systems equally well. The previous two figures show RNA yield and purity data from samples that were processed manually. In the following figure, RNA was isolated using a Biomek® 2000 Workstation (Beckman Coulter), and samples were tested for the presence of the bovine viral diarrhea virus (BVDV) using qRT-PCR.

BVDV is a major problem worldwide that results in economic losses in the beef and dairy industries. Blood and milk are ideal sample sources to test animals for BVDV. The MagMAX™-96 Blood Kit was tested for reproducibility and lack of sample cross contamination by collecting bovine blood from an animal known to be BVDV-infected and from an animal known to be free of BVDV infection. 50 µL samples of blood were distributed to wells of a 96-well processing plate, and RNA was isolated using the MagMAX™-96 Blood RNA Isolation Kit. Samples were processed using a Biomek 2000 Workstation. Processing a fully-loaded plate requires ~1 hour. The purified RNA was subsequently used for qRT-PCR targeting BVDV RNA.

The BVDV RNA target amplification results corresponded perfectly to the BVDV negative and positive sample distribution in the 96-well plate (Figure 4). BVDV RNA was recovered reproducibly, and no sample cross contamination was observed. In addition, BVDV target amplification was highly consistent, with an average C_t of 23.08 ± 0.3.

Figure 4 qRT-PCR to Detect Viral RNA in Bovine Blood RNA Isolated with a Robotic Liquid Handling System. 50 µL bovine blood samples, from a single BVDV-positive animal (darker wells) and a single BVDV-negative animal (paler wells) were processed for RNA isolation using the MagMAX™-96 Blood RNA Isolation Kit on a Biomek® 2000 Workstation. Samples were then subjected to diagnostic qRT-PCR, and Ct values are shown for samples that had detectable amounts of BVDV. Note that all BVDV-positive samples were detected, and no sample cross-contamination was seen.

BVDV	1	2	3	4	5	6	7	8	9	10	11	12
A	*	*	*	22.91	*	*	*	23.10	*	*	*	22.77
B	*	22.92	*	*	*	22.74	*	*	*	22.91	*	*
C	*	*	*	23.08	*	*	*	22.69	*	*	*	22.61
D	*	23.13	*	*	*	23.17	*	*	*	23.03	*	*
E	*	*	*	23.26	*	*	*	23.04	*	*	*	22.77
F	*	23.18	*	*	*	23.32	*	*	*	22.64	*	*
G	*	*	*	23.64	*	*	*	23.41	*	*	*	23.11
H	*	23.13	*	*	*	23.68	*	*	*	23.37	*	*

Avg Ct: 23.08, Standard Deviation: 0.30

* Not Detected

Extending functionality of the MagMAX™-96 Blood Kit

Cultured cells and small tissue samples

The MagMAX™-96 Blood RNA Isolation Kit is optimized for RNA isolation from blood and milk samples. It can also be used for RNA isolation from cultured cells (25 cells to 2 x 10^6 cells) and tissue lysates (≤5 mg tissue).

Isolation of total nucleic acid

The MagMAX™-96 Blood RNA Isolation Kit can be used for total nucleic acid isolation by omitting the DNase treatment. Without DNase treatment, however, the RNA Binding Beads may clump, which could result in inefficient DNA/RNA elution. If low and/or inconsistent yield is observed, using less sample or using more RNA Binding Beads may alleviate the problem. The necessary protocol adjustments vary considerably among different samples and must be determined experimentally.

Kit components and storage conditions

The MagMAX™-96 Blood RNA Isolation Kit contains reagents to isolate RNA from 96 samples.

Amount	Component	Storage
1	Processing Plate & Lid	room temp
32 mL	Lysis/Binding Solution Concentrate Add 16 mL 100% isopropanol before use.	room temp†
36 mL	Wash Solution 1 Concentrate Add 12 mL 100% isopropanol before use.	room temp
55 mL	Wash Solution 2 Concentrate Add 44 mL 100% ethanol before use.	room temp
9 mL	Elution Buffer	room temp
6 mL	MagMAX™ TURBO™ DNase Buffer	4°C or room temp
1.1 mL	RNA Binding Beads	4°C†
1.1 mL	Lysis/Binding Enhancer	–20°C
110 µL	TURBO DNase™ Enzyme(10 U/µL)	–20°C

† Do not freeze.

Required materials not provided with the kit

Lab equipment and supplies

- General laboratory equipment including vortex mixer, microcentrifuge, pipettors, and RNase-free pipette tips
- Magnetic stand for 96-well plates: We recommend either of the Ambion 96-well magnetic stands (Cat. nos. AM10050, AM10027) for their high strength magnets and quality design.
- (Optional but recommended) Multichannel pipettor
- Orbital shaker for 96-well plates such as the Barnstead/Lab-Line Titer Plate Shaker (VWR #57019-600 or Fisher #14-271-9)
- If you process fewer than 96 samples at a time, you will need additional polystyrene U-bottom 96-well plates and lids.

Reagents

- 100% ethanol, ACS grade or higher quality
- 100% isopropanol, ACS grade or higher quality

MagMAX-96 Blood RNA isolation procedure

Reagent and equipment preparation

1. RNase precautions

   Lab bench and pipettors

   Before working with RNA, it is always a good idea to clean the lab bench and pipettors with an RNase decontamination solution (e.g., Ambion RNaseZap® Solution).

   Gloves and RNase-free technique

   Wear laboratory gloves for this procedure; they protect you from the reagents, and they protect the RNA from nucleases that are present on skin.

   Use RNase-free pipette tips to handle the kit reagents, and avoid putting used tips into the reagent containers.

2. Determine maximum 96-well plate shaker settings

   For larger volumes

   Place 210 µL water in the wells of a 96-well plate and use it to determine the maximum shaker setting that can be used with your orbital shaker without sample spillage. This maximum shaker speed will be used for most steps of the procedure.

   For smaller volumes

   Place 50 µL of water in the wells of a 96-well plate and use it to determine the maximum shaker setting that can be used with your orbital shaker without sample spillage. Use this speed for the bead drying and RNA elution steps.

3. Before using the kit, complete Lysis/Binding Solution, and Wash Solutions 1 and 2

   a. Add 16 mL 100% isopropanol to the bottle labeled Lysis/Binding Solution Concentrate and mix well.

      The mixture is called Lysis/Binding Solution in these instructions.

   b. Add 12 mL 100% isopropanol to the bottle labeled Wash Solution 1 Concentrate and mix well.

      The resulting mixture is called Wash Solution 1 in these instructions.

   c. Add 44 mL 100% ethanol to the bottle labeled Wash Solution 2 Concentrate and mix well.

      The resulting mixture is called Wash Solution 2 in these instructions.

   d. Mark the labels of the solutions to indicate that the isopropanol or ethanol was added.

      Store the solutions at room temperature.

4. Prepare Bead Mix

   Each isolation reaction requires 20 µL of Bead Mix. Although the mixture is tested to be stable at 4°C for up to 2 weeks, we recommend preparing Bead Mix on the day it will be used.

   a. Vortex the RNA Binding Beads at moderate speed to form a uniform suspension before pipetting.

b. Prepare Bead Mix by combining the volumes of RNA Binding Beads and Lysis/Binding Enhancer shown in the table below appropriate for the number of isolation reactions to be performed that day. Mix thoroughly.

   We recommend including ~10% overage to cover pipetting error when preparing the Bead Mix.

Component	Per reaction	~100 reactions
RNA Binding Beads	10 µL	1.1 mL
Lysis/Binding Enhancer	10 µL	1.1 mL

c. Place the prepared Bead Mix on ice until it is needed.

Sample collection

For the highest quality RNA, use whole blood or milk samples that have not been frozen. RNA can be recovered from frozen samples, but its quality is typically compromised as a result of the freeze-thawing process.

Whole blood

Collect whole blood into any of the following anticoagulants and use it immediately for RNA isolation:

- sodium EDTA
- potassium EDTA
- heparin
- sodium citrate

If long-term storage of whole blood is required, we recommend one of the following methods:

- Collect blood into Tempus™ Blood RNA Tubes (Cat. no. 4342792) and use the MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit (Compatible with Tempus™ Blood RNA Tubes) (Cat. no. 4451893) for RNA isolation.
- Collect blood into PAXgene® Blood RNA Tubes (available from PreAnalytiX, Cat. no. 762165) and use the MagMAX™ for Stabilized Blood Tubes RNA Isolation Kit (Compatible with PAXgene® Blood RNA Tubes) (Cat. no. 4451894) for RNA isolation.

Milk

Milk samples can be stored at 4°C for up to 2 weeks for use in the MagMAX™-96 Blood RNA Isolation procedure.

Sample lysis and initial nucleic acid purification

These instructions are appropriate for isolating total RNA and viral RNA from up to 50 µL of whole blood or milk. For larger samples, additional reagents are required.

Note: 96 samples can be processed at once, but with additional polystyrene U-bottom 96-well plates and lids, RNA can also be efficiently isolated from fewer samples.

1. Add 50 µL sample to 130 µL Lysis/Binding Solution and mix by pipetting up and down at least five times.
   a. For each sample, add 130 µL Lysis/Binding Solution to a well of the Processing Plate provided with the kit.

b. Add a 50 µL sample of whole blood or milk to the Lysis/Binding Solution in the plate.

IMPORTANT! It is vital to pipet the blood/lysis buffer mixture for complete mixing at this point to ensure proper lysis of blood cells.

c. Shake the plate for 2 min on an orbital shaker at the maximum speed for larger volumes identified at the beginning of the procedure.

2. Add 20 µL of Bead Mix and shake for 5 min

   a. Vortex the Bead Mix at moderate speed to create a uniform suspension before pipetting.

   b. Add 20 µL of Bead Mix to each sample.

   c. Shake the plate for 5 min at the maximum speed for larger volumes identified in the previous section.

      During this incubation, cells are completely lysed and nucleic acids bind to the RNA Binding Beads.

3. Magnetically capture the RNA Binding Beads and carefully discard the supernatant

   a. Move the Processing Plate to a magnetic stand to capture the RNA Binding Beads. Leave the plate on the magnetic stand for at least 3–5 min.

   b. Carefully aspirate and discard the supernatant without disturbing the beads, and remove the Processing Plate from the magnetic stand.

IMPORTANT! To obtain pure RNA, it is important to completely remove the supernatant at this step.

4. Wash with 150 µL Wash Solution 1

   This wash removes residual nucleases, preserving RNA integrity.

   a. Add 150 µL Wash Solution 1 to each sample and shake for 1 min at the maximum speed for larger volumes.

   b. Capture the RNA Binding Beads on a magnetic stand. Leave the plate on the stand until the mixture becomes transparent, indicating that capture is complete. The capture time depends on the magnetic stand used. Using the Ambion 96-Well Magnetic-Ring Stand, the capture time is ~1 min.

   c. Carefully aspirate and discard the supernatant without disturbing the beads, and remove the Processing Plate from the magnetic stand.

   d. (Optional) Repeat above steps to wash a second time with Wash Solution 1. This second wash will improve RNA purity in samples that are rich in proteins, lipids, or other PCR inhibitors. It may not be necessary for samples that do not contain these contaminants.

5. Wash with 150 µL Wash Solution 2

   a. Add 150 µL Wash Solution 2 to each sample and shake for 1 min as above.

   b. Capture the RNA Binding Beads on a magnetic stand as above.

   c. Carefully aspirate and discard the supernatant without disturbing the beads, and remove the Processing Plate from the magnetic stand.

TURBO™ DNase treatment and final clean-up

1. Prepare Diluted TURBO™ DNase

   At room temperature, combine the volumes of MagMAX TURBO DNase Buffer with TURBO DNase enzyme shown in the table below appropriate for the number of samples being processed plus ~10% overage to cover pipetting error. Mix thoroughly. Use the Diluted TURBO DNase solution immediately once it is prepared.

Component	per reaction	~100 reactions
MagMAX™ TURBO™ DNase Buffer	49 µL	5.4 mL
TURBO™ DNase Enzyme	1 µL	110 µL

2. Add 50 µL of Diluted TURBO DNase solution and shake for 5 min

   This step removes genomic DNA from the RNA.

   a. Add 50 µL Diluted TURBO DNase solution to each sample.

   b. Shake the plate for 5 min at room temp at the maximum speed for larger volumes identified at the beginning of the procedure.

3. Add 130 µL Lysis/Binding Solution and shake for 3 min to rebind the RNA

   Add 130 µL of Lysis/Binding Solution to each sample and shake for 3 min at the maximum speed for larger volumes.

   In this step, the RNA is bound to the RNA Binding Beads again.

4. Magnetically capture the RNA Binding Beads and carefully discard the supernatant

   a. Capture the RNA Binding Beads on a magnetic stand as in the previous steps.

      Using the 96-Well Magnetic-Ring Stand, the capture time is ~1 min.

   b. Carefully aspirate and discard the supernatant without disturbing the beads, and remove the Processing Plate from the magnetic stand.

5. Wash the RNA Binding Beads twice with 150 µL Wash Solution 2

   These washes remove the TURBO DNase treatment reagents and any other contaminants.

   a. Add 150 µL Wash Solution 2 to each sample and shake for 1 min as in the previous step.

   b. Capture the RNA Binding Beads on a magnetic stand as in the previous steps.

   c. Carefully aspirate and discard the supernatant without disturbing the beads, and remove the Processing Plate from the magnetic stand.

   d. Repeat above steps to wash the RNA Binding Beads with 150 µL Wash Solution 2 a second time.

6. Dry the beads by shaking for 2 min

   Move the Processing Plate to the shaker and shake vigorously for 2 min at the maximum speed for lower volumes identified at the beginning of the procedure.

   This dries the beads, removing residual ethanol which otherwise could impair RT-PCR efficiency.

7. Elute the RNA in 30–50 µL Elution Buffer

   a. Add 30–50 µL Elution Buffer to each sample and shake for 3 min at the maximum speed for lower volumes identified at the beginning of the procedure.

      Note: The elution volume is somewhat flexible; if desired, RNA can be eluted in as little as 20 µL Elution Buffer. Likewise, the elution volume can be >50 µL, however you may run short of the Elution Buffer supplied with the kit if you use >50 µL for every reaction.

   b. Capture the RNA Binding Beads on a magnetic stand as in the previous step. The purified RNA will be in the supernatant.

   c. Transfer the supernatant, which contains the RNA, to a nuclease-free container appropriate for your application.

   d. Store the purified RNA at –20°C.

Assessing RNA yield and purity

RNA yield

- Spectrophotometry

  The concentration of an RNA solution can be determined by measuring its absorbance at 260 nm (A_{260}) using a spectrophotometer. With a traditional spectrophotometer, dilute an aliquot of the RNA 1:50–1:100 in TE (10 mM Tris-HCl pH 8, 1 mM EDTA), and read the absorbance. (Be sure to zero the spectrophotometer with the TE used for sample dilution.) The buffer used for dilution need not be RNase-free, since slight degradation of the RNA will not significantly affect its absorbance.

  NanoDrop spectrophotometers are more convenient—no dilutions or cuvettes are needed, just measure 1.5 µL of the RNA sample directly.

  To determine the RNA concentration in µg/mL, multiply the A_{260} by the dilution factor and the extinction coefficient (1 A_{260} = 40 µg RNA/mL).

  A_{260} x dilution factor x 40 = µg RNA/mL

  Be aware that any contaminating DNA in the RNA prep will lead to an overestimation of yield, since all nucleic acids absorb at 260 nm.

- Fluorometry

  If a fluorometer or a fluorescence microplate reader is available, Molecular Probes' RiboGreen® fluorescence-based assay for RNA quantitation is a convenient and sensitive way to measure RNA concentration. Follow the manufacturer's instructions for using RiboGreen.

RNA quality

- Microfluidic analysis

  The Agilent 2100 Bioanalyzer™ instrument with Caliper's RNA LabChip® Kits provides better qualitative data than conventional gel analysis for characterizing RNA. When used with the Ambion RNA 6000 Ladder (Cat. no. AM7152), this system can provide a fast and accurate size distribution profile of RNA samples. Follow the manufacturer's instructions for performing the assay.

  The 28S to 18S rRNA ratio is often used as an indicator of RNA integrity. Total RNA isolated from unfrozen whole blood using this kit usually has a 28S to 18S rRNA ratio of >0.8.

Using a Bioanalyzer™ instrument, the RIN (RNA Integrity Number) can be calculated to further evaluate RNA integrity. A new metric developed by Agilent, the RIN analyzes information from both rRNA bands, as well as information contained outside the rRNA peaks (potential degradation products) to provide a fuller picture of RNA degradation states. Search for "RIN" at Agilent's website for information:

http://www.chem.agilent.com

- Spectrophotometry

  An effective measure of RNA purity is the ratio of absorbance readings at 260 and 280 nm. The total RNA isolated with this kit should have an A_{260}/A_{280} ratio of 1.8–2.1. However, RNA with absorbance ratios outside of this range may still function well for qRT-PCR or other amplification-based downstream applications.

Troubleshooting

Variation in RNA yield between wells

The total RNA yield should be fairly uniform between wells of a 96-well plate with the same sample type; however, RNA recovery from different samples types such as blood or milk may vary considerably. The following troubleshooting suggestions may be helpful if large variations in RNA yield from the same sample type are observed.

RNA Binding Beads were not fully resuspended/dispersed

In general, the RNA Binding Beads will disperse more easily when the temperature of the mixture is warmer than ~20°C (68°F).

a. Make sure the RNA Binding Beads are fully resuspended before pipetting them into the Processing Plate at the start of the procedure.

b. Make sure that the RNA Binding Beads are fully resuspended in Elution Buffer to efficiently elute the RNA from the beads.

   If the RNA Binding Beads aggregate or fail to disperse during the elution step, it may improve RNA yield to place the Processing Plate in a 70°C incubator for 5 min and to repeat the 3 min shaking incubation before proceeding to step 7.b. on page 12.

   In subsequent experiments using sample types with bead clumping problems, you can preheat the Elution Buffer to 70–80°C before adding it to the samples in step 7.a. on page 12. to facilitate dispersion of the beads.

c. Do not overdry the beads before eluting. If the beads were inadvertently overdried, extend the shaking time in step 7. on page 12 to 10 min to rehydrate the beads.

RNA Binding Beads were unintentionally lost

Since the basis of this procedure is to immobilize RNA on RNA Binding Beads, any loss of beads during the procedure will result in loss of RNA. Avoid aspirating RNA Binding Beads when removing supernatant from the captured beads. To determine whether RNA Binding Beads have been inadvertently aspirated with supernatant, it may be helpful to collect all supernatants in a single reservoir. Observe the color of the collected supernatant, if RNA Binding Beads are in the supernatant, they will tint the solution light brown.

To prevent aspiration of RNA Binding Beads in subsequent experiments, observe the following precautions:

- Use sufficient magnetic capture time.
- Aspirate supernatant slowly.
- Keep pipette tip openings away from the captured RNA Binding Beads when aspirating supernatant.

DNA contamination

RNA Binding Beads were not fully resuspended/dispersed

If the RNA Binding Beads aggregate or fail to disperse after you add the diluted TURBO DNase solution in step 2. on page 12, it may improve DNA digestion to place the Processing Plate in a 37°C incubator for 5 min before the 5 min shaking incubation.

In subsequent experiments using sample types with bead clumping problems, you can preheat the the Diluted TURBO DNase solution to 37°C for 5 min before adding it to the samples in step 7.a. on page 12, to facilitate dispersion of the beads.

Ambient temperature is below ~20°C (68°F)

If the ambient temperature in your lab is colder than ~20°C (68°F), we recommend increasing the TURBO DNase digestion incubation time in step 2. on page 12 to 10 min for better genomic DNA removal.

Low viral RNA detection sensitivity

Difficulty in detecting viral RNA by quantitative RT-PCR can be caused either by an insufficient amount of target or by the presence of impurities that inhibit RT-PCR.

Impurities that inhibit RT-PCR

Most impurities will cause a shift in UV absorbance that can be seen by comparing a sample's UV absorbance spectrum to that of a control RNA. Any distortion in the shape of UV spectrum indicates that there are impurities in the eluted RNA. For example, protein absorbs at 280 nm, which can result in a low A_{260}/A_{280} ratio. Salt contamination may cause a peak at 230 nm.

- Include the second (optional) wash with Wash Solution 1 in step 4.d. on page 12.

  This second wash helps to remove protein and other RT-PCR inhibitors from samples.
- Remove supernatants from captured RNA Binding Beads thoroughly to avoid salt contamination.

  The Lysis/Binding and Wash Solutions contain signigficant amounts of salts, completely remove supernatants from RNA Binding Beads to avoid salt carryover.

RNA Binding Bead carryover

If RNA Binding Beads are carried over into the eluate containing the RNA, they will cause the solution to be light brown in color. A small quantity of beads in the sample does not inhibit RT reactions or RT-PCR.

- See section "RNA Binding Beads were unintentionally lost" on page 15 for suggestions for avoiding bead carryover.
- To remove RNA Binding Beads from RNA samples, place the plate or tubes on a magnetic stand to capture the beads for ~1 min. Then transfer the RNA solution(s) to a fresh nuclease-free plate or tubes.

A Supplemental Information

Related products available from Life Technologies

RNase*Zap*® Solution Cat. nos. AM9780, AM9782, AM9784	RNase*Zap*® RNase Decontamination Solution is simply sprayed, poured, or wiped onto surfaces to instantly inactivate RNases. Rinsing twice with distilled water will eliminate all traces of RNase and RNase*Zap*® Solution.
RNase-free Tubes & Tips	RNase-free tubes and tips are available in most commonly used sizes and styles. They are guaranteed RNase- and DNase-free.
96-well Magnetic-Ring Stand Cat. no. AM10050	The Ambion 96-well Magnetic-Ring Stand features 96 powerful ring-shaped magnets arranged to cradle each well of a 96-well plate for quick, thorough bead capture. Captured magnetic beads form evenly distributed donut-shaped pellets with a large hole in the center. This capture pattern facilitates both supernatant removal and subsequent bead resuspension. The stand is suitable for high throughput applications conducted with multichannel pipettors or with robotic liquid handlers. However, because the pellets will be evenly distributed around the edge of the wells, it may require practice for efficient manual removal of supernatants.
Magnetic Stand-96 Cat. no. AM10027	The Ambion Magnetic Stand-96 has powerful magnets positioned to capture beads to one side of the well. This capture pattern makes it very easy to remove supernatants manually without disturbing the beads, and therefore may be preferred by beginning users. In some applications, however, pellets formed with the Magnetic Stand-96 may be difficult to resuspend. If this occurs, we recommend the 96-well Magnetic-Ring Stand (Cat. no. AM10050).
Electrophoresis Reagents	Life Technologies offers gel loading solutions, agaroses, acrylamide solutions, powdered gel buffer mixes, nuclease-free water, and RNA and DNA molecular weight markers for electrophoresis.

Quality control

Functional testing

The kit is tested functionally by isolating RNA from 50 ?L of fresh human blood spiked with a 1 kb control RNA and an armored HIV RNA control using the protocol in this user guide. RNA recovery is determined by UV absorbance measurements and quantitative RT-PCR.

Nuclease testing

Relevant kit components are tested in the following nuclease assays:

RNase activity

Asample is incubated with labeled RNA and analyzed by PAGE.

Nonspecific endonuclease activity

A sample is incubated with supercoiled plasmid DNA and analyzed by agarose gel electrophoresis.

Exonuclease activity

A sample is incubated with labeled double-stranded DNA, followed by PAGE analysis.

Protease testing

A sample is incubated with protease substrate and analyzed by fluorescence.

B KingFisher MagMAX™-96 Blood RNA Isolation

KingFisher MagMAX-96 Blood RNA isolation procedure overview

The MagMAX™-96 Blood RNA Isolation procedure can be adapted for use with Thermo Electron's KingFisher (for 1–24 samples per run) and KingFisher 96 (for 1–96 samples per run) Magnetic Particle Processors.

The KingFisher processors completely automate the nucleic acid isolation process; here is a quick overview of how it works:

1. Pipet MagMAX™-96 Blood RNA Isolation Kit reagents into a KingFisher 200 µL plate(s) and insert the plate(s) into the KingFisher or KingFisher 96 instrument.

Row /Plate	Volume	Reagent(s)
A	130 µL	Lysis/Binding Solution (isopropanol added)
	50 µL	Sample
	20 µL	Bead Mix
	200 µL	total volume
B	150 µL	Wash Solution 1
C	150 µL	Wash Solution 2
D	50 µL	Diluted TURBO DNase
E	150 µL	Wash Solution 2
F	150 µL	Wash Solution 2
G	50 µL	Elution Buffer

2. Choose the MagMAX Blood program using the arrow keys and start the program by pressing the START button. The approximately 25 min process is described below:
3. Total RNA is bound to RNA Binding Beads in row A (plate A the KingFisher 96) containing sample, Lysis/Binding Solution, and Bead Mix.
4. The RNA Binding Beads are collected and released into the first Wash 1 Solution in row B (plate B).
5. The RNA Binding Beads are collected and released into the first Wash 2 Solution in row C (plate C).
6. The RNA Binding Beads are collected and released into the diluted TURBO DNase in row D (plate D).
7. The machine pauses and 130 µL of lysis/binding solution is added to row D (plate D) by the user. Press the START button to continue. The machine mixes and rebinds the RNA.
8. The RNA Binding Beads are collected and released into the second Wash 2 Solution in row E (plate E).

9. The RNA Binding Beads are collected and released into the third Wash 2 Solution in row F (plate F).
10. The RNA Binding Beads are collected and lifterd outside the wells of row F (plate F) to dry for 1 min.
11. The RNA Binding Beads are released into Elution Buffer in row G (plate G).

The used RNA Binding Beads are collected and returned to row B (plate B leaving the RNA in the Elution Buffer in row G [plate G]).

C

Safety

General Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:
Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.

- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.).
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of

according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:
- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/ 29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:
- Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Documentation and Support

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/sds**

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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