For general laboratory use.

MagNA Pure LC RNA Isolation Kit – High Performance

Version: 14

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Kit for isolation of total RNA from mammalian whole blood, blood cells, and cultured cells

Cat. No. 03 542 394 001 1 kit
up to 192 isolations

Store the kit at +15 to +25°C.

1. General Information

1.1. Contents

Vial / Bottle	Cap	Label	Function / Description	Content
1	black	Wash Buffer I	for removing PCR inhibitors	4 bottles,100 ml each
2	blue	Wash Buffer II	for removing salts, proteins, etc.	4 bottles,100 ml each
3	green	Lysis/Binding Buffer	for cell lysis and binding of RNA	2 bottles, 100 ml each
4	black	Magnetic Glass Particles	for binding RNA	6 vials, MGP suspension
5a	white	DNase	for DNA digestion	6 vials, lyophilizate
5b	white	DNase Incubation Buffer	for reconstitution of DNase	1 bottle, 100 ml
6	red	Isopropanol	for rebinding of RNA	1 bottle, 100 ml
7a	pink	Proteinase K	for digestion of proteins	6 glass vials, lyophilizate
7b	colorless	Proteinase K Buffer	for reconstitution of Proteinase K	1 bottle, 100 ml
8	yellow	Elution Buffer	▪ for elution of RNA ▪ for dilution of eluate (optional)	1 bottle, 100 ml

The Lysis/Binding Buffer contains a blue ingredient needed for clot detection during automated RNA isolation by the MagNA Pure LC 2.0 Instrument.

The bottles of the Wash Buffer I and MGPs have both black caps, although the color-coding of MagNA Pure LC Software and Positioning Frames is referring to a caramel cap for the MGPs.

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +15 to +25°C, the kit is stable through the expiration date printed on the label.

Storage Conditions (Working Solution)

Solution	Storage
Proteinase K	Reconstituted Proteinase K can be stored in a refrigerator at +2 to +8°C for up to 4 weeks, or at −15 to −25°C for up to one year.
Lysis/Binding Buffer	After adding DTT, store the Lysis/Binding Buffer with 1% DTT at +15 to +25°C and use it within 4 to 6 months.
DNase Solution	Reconstituted DNase can be stored in a refrigerator at +2 to +8°C for up to 4 weeks, or at −15 to −25°C for up to one year.

1.3. Additional Equipment and Reagent required

- MagNA Pure LC 2.0 Instrument

Standard laboratory equipment
- Pipettes and nuclease free, aerosol-preventive tips to pre-dispense samples into the sample cartridge
- Centrifuge and suitable nuclease free reaction tubes
- Roller incubator to agitate sample for lysis of erythrocytes and pre-isolation of WBCs
- Vortex mixer
- DTT*

Optional:
- Red Blood Cell Lysis Buffer* (for pre-isolation of WBCs)
- PBS* (for pre-isolation of blood cells, primary cells, and cultured cells)
- Vacutainer CPT tube (BD Diagnostics; for pre-isolation of PBMCs)
- hemocytometer (*e.g.*, Neubauer device with counting chambers; for pre-isolation of blood cells)

1.4. Application

Preparation of total RNA from mammalian whole blood, blood cells, or cultured cells using the MagNA Pure LC 2.0 Instrument. The purified RNA can be used for RT-PCR with LightCycler® Instruments, standard thermal block cycler RT-PCR, northern blot analysis, and other typical downstream applications in gene-expression analysis. Purified RNA is free of any PCR inhibitors.

Product Description

The MagNA Pure LC RNA Isolation Kit – High Performance is used for automated isolation of total RNA from up to 200 µl mammalian whole blood or up to 10^6 blood cells or cultured cells.

The kit is designed to process up to 192 samples in batches of 32 (depending on the protocol). If you process fewer than 32 samples at a time, some reagent will be wasted and the remaining reagent may not be enough to process the number of samples listed above.

1.5. Preparation Time

Assay Time

MagNA Pure LC 2.0 Instrument set-up	15 minutes total
Instrument run time	Approximately 90 minutes when processing 1 to 32 samples, using either the 'RNA HP Blood' or 'RNA HP Blood_external_lysis' purification protocol.
	Approximately 150 minutes when processing 1 to 32 samples, using the 'RNA HP Cells' purification protocol.

No hands-on time is required after external lysis of the blood or blood cell samples and MagNA Pure LC 2.0 Instrument set-up. Extra hands-on time is required for the manual external lysis procedure.

2. How to Use this Product

2.1. Before you Begin

Sample Materials

To obtain optimal results in downstream procedures, especially in LightCycler® System RT-PCR, do not process samples larger than this kit is designed to handle. Optimal amount of sample material are as follows:

- Up to 200 µl of whole mammalian blood
- White blood cells (WBCs) isolated from up to 200 µl of normal mammalian blood containing no more than
- 1×10^6 WBCs.
- Peripheral blood mononuclear cells (PBMCs) isolated from up to 400 µl of normal mammalian blood containing no more than 1×10^6 PBMCs.
- Up to 1×10^6 cultured cells.

Never use more sample material than recommended in the above guidelines. Using greater cell numbers than this kit is designed to handle can lead to clumping and loss of MGPs during robotic pipetting and significantly reduce the performance and RNA yield of the isolation process.

Do not process more than 1×10^6 WBCs or PBMCs in a single sample. Please note that the actual concentration of WBCs and PBMCs in human blood may differ from the values given above. For best results, count the WBCs and PBMCs with a manual hemocytometer before attempting to purify RNA at the upper limit of cell volumes such as 200 µl whole blood. Please note that automatic counting systems from different suppliers can produce cell counts that may not be in agreement with cell counts using a manual hemocytometer counts.

It is important to consider that different mammalian species may have different concentrations of blood cells. For some species, this means you may need to use a smaller sample of blood to keep the cell numbers within the above guidelines.

⚠ ***Treat all samples as potentially infectious.***

Control Reactions

Positive and negative control sample materials

Always run appropriate controls with the samples for use when performing downstream applications with the eluted RNA samples (*e.g.,* by LightCycler® System RT-PCR assays).

Monitor both, the process of sample preparation and subsequent downstream applications by using the following control sample materials:

- Positive Control using a sample material that is known to be positive for your target.
- Negative Control using a sample material that is known to be negative for your target.
- Internal Control, by adding a defined amount of a control template to all samples to be purified.

The Internal Control (IC) is added prior to the purification step and then co-purified and amplified with your target of interest from the specimen in the same RT-PCR reaction. The IC concept is especially useful for enzyme based amplification processes such as RT-PCR, because efficiency of the RT-PCR process might be reduced by inhibitors present in the purified sample material. In addition, the Internal Control is used to compensate for possible losses of your target during purification.

For quantification assays using LightCycler® Instruments, use an in vitro transcribed RNA molecule with primer-binding sites identical to those of your target sequence, but having a unique probe-binding region that differentiates the IC from the target-specific amplicon. Discriminate signals derived from your target and the IC by performing a dual HybProbe assay. For detailed information regarding the IC concept combination with the LightCycler® Carousel-Based Systems, read LightCycler® 2.0 Instrument Technical Note 12/2000 "Absolute Quantification with External Standards and Internal Control" available at http://technical-support.roche.com.

General Considerations

Handling requirements

⚠ ***Wash Buffer I (bottle 1) and Lysis/Binding Buffer (bottle 3) contain guanidinium salts which are hazardous irritants. Do not allow the Wash Buffer I or Lysis/Binding Buffer to come in contact with skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water. If necessary, immediately contact your laboratory supervisor, and seek medical assistance. When spilling these reagents, dilute the spill with large amounts of water before attempting to clean up the spill.***

⚠ ***Do not allow Wash Buffer I or the Lysis/Binding Buffer to mix with sodium hypochlorite (bleach) solution. This mixture can produce a highly toxic gas.***

⚠ ***Do not pool reagents from different MagNA Pure LC reagent lots or from different bottles of the same lot.***

⚠ ***Do not use a kit after its expiration date has passed.***

Safety Information

Laboratory procedures

- Handle all samples as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator must optimize pathogen inactivation by the Lysis/Binding Buffer or take appropriate measures, according to local safety regulations.
- Do not eat, drink or smoke in the laboratory work area.
- Do not pipette by mouth.
- Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease-free pipet tips only, to remove aliquots from reagent bottles. Use the general precautions described in the literature.
- Wash hands thoroughly after handling samples and reagents.
- Finish each phase of the PCR/RT-PCR workflow before proceeding to the next phase. For example, you should finish PCR/RT-PCR sample preparation before starting PCR/RT-PCR set-up. Sample preparation, PCR/RT-PCR set-up and the PCR/RT-PCR run itself should also be performed in separate locations.

Waste handling

- Discard unused reagents and waste in accordance with country, federal, state, and local regulations.
- Safety Data Sheets (SDS) are available online on dialog.roche.com, or upon request from the local Roche office.

Working Solution

Before starting the procedure, prepare the working solutions as described below.

All other solutions are ready-to-use.

Incubate buffers at +15 to +25°C before use. If you use the reagents at temperatures outside the recommended range, the kit may not work well.

All buffers are clear. Do not use a buffer if it contains a precipitate. If a precipitate is visible, place the bottle at +37°C and mix from time to time until the precipitate is completely dissolved. Do not warm the buffer longer at 37°C than is needed for dissolution of the precipitate. Before using it, make sure the buffer is +15 to +25°C.

Do not store the Proteinase K, DNase, and the MGP suspension in Reagent Tubs. All other reagents remaining in the Reagent Tubs after completion of the run can be used for the next run if performed on the same day. Longer storage periods are not recommended.

Reagent	Preparation / Comments	Storage
Lysis/Binding Buffer (bottle 3)	*Necessarily required for lysis of blood cells (WBCs/PBMCs) and primary cells. The buffer is also used in the instrument by the robotic nozzlehead to lyse primary cells and cultured cells such as K562 or HeLa cells, and after DNase digestion, to rebind RNA to the MGPs in the presence of isopropanol.* Add 2 ml of a freshly prepared 50% DTT* stock solution to 100 ml Lysis/Binding Buffer (to reach a final conc. of 1% DTT).	▪ Store Lysis Buffer at +15 to +25°C. ***After addition of DTT, use the Lysis/Binding Buffer up within 4 to 6 months.***
Magnetic Glass Particles	The MGP suspension (bottle 4) must be mixed thoroughly. Vortex immediately before use to produce a homogeneous suspension. The beads tend to sediment during storage. *For best results, add the MGPs to the instrument just before starting the run (to minimize sedimentation). Always use the exact amount of MGPs recommended by the software.*	Store MGPs at +15 to +25°C. ***Do not leave the MGP suspension uncovered in the bottle or in the Reagent Tub, as evaporation of alcohol might lead to suboptimal purification.***
DNase Solution	Reconstitute each bottle of DNase (bottle 5a) with 10 ml DNase Incubation Buffer (bottle 5b). Close the bottle and mix well. *One bottle is sufficient for 32 samples.*	Once reconstituted the DNase is stable for 1 month at +2 to +8°C or up to 12 months at -15 to -25°C.
Proteinase K	Reconstitute each bottle Proteinase K (vial 7a) by adding 3.0 ml Proteinase K Buffer II (vial 7b). After full solubilization, add additional 2 ml of the buffer to reach the final volume. Close the vial and mix well. ***After dissolving Proteinase K in Proteinase K Buffer II, the solution might appear turbid. This is caused by stabilizing components added to Proteinase K. This appearance of the Proteinase K solution has no impact on the functionality of the enzyme.*** *One bottle is sufficient for 32 samples.*	Once reconstituted the Proteinase K is stable for 1 month at +2 to +8°C or up to 12 months at -15 to -25°C.

2.2. Protocols

Pre-Isolation Steps

General Remarks

Pre-isolation steps are required for the “RNA HP Blood_external_lysis” and “RNA HP Cells” purification protocol, which include a manual sample lysis step.

The protocols for whole blood and blood cells were developed with human blood.
Store the lysate at-60 to -80°C when RNA isolation is postponed or further processing on the instrument is needed.

Whole Blood

- If you use the ‘RNA HP Blood’ protocol, no sample preparation is necessary: Transfer 20 - 200 µl whole blood directly into the Sample Cartridge.
- If you use the ‘RNA HP Blood_external_lysis’ protocol, add 700µl Lysis/Binding Buffer (bottle 3) to the primary sample (20 - 200 µl) and mix well. Then, either transfer the lysate directly to the Sample Cartridge or store it frozen at -60 to -80°C for later use (stable for at least 3 months).

If blood samples could contain an unusual high blood cell number (≥10^4 cells/ml), count the cells and use correspondingly less sample volume to avoid overloading.
Do not use frozen blood, because this could lead to degradation of RNA.

WBCs

For manual pre-isolation of WBCs from up to 200 µl fresh, stabilized mammalian whole blood follow the procedure below.

You will need two volumes of Red Blood Cell Lysis Buffer for every volume of blood processed.

1. -Pipet two volumes of up to 400 µl of RBC Lysis Buffer into a tube and warm it to room temperature (+15 to +25°C).
   - Add one volume of up to 200 µl of blood to the RBC Lysis Buffer and mix gently by inverting the sample 5 times manually.
   -Incubate this sample mixture for 10 minutes on a roller incubator at +15 to +25°C.
   ⚠ ***After incubation the solution should be clear.***

2. -Centrifuge the sample for 5 minutes at 600 ×*g* at +15 to +25°C.
   -Discard supernatant.
   ⚠ ***A white pellet should be visible.***

3. Resuspend the cell pellet in 100 µl PBS.

4. -Add 100 µl Lysis/Binding Buffer (bottle 3)
   -Mix thoroughly by vortexing.
   ⚠ ***Use only Lysis/Binding Buffer containing 1% DTT.***

5. Transfer each lysate into one well of the sample cartridge.

6. Place the sample cartridge on the reagent/sample stage, and start the ‘RNA HP Cells’ protocol.

Peripheral blood mononuclear cells (PBMCs)

For the manual pre-isolation of PBMCs from up to 400 µl fresh, stabilized whole blood follow the procedure below.

1. Collect up to 8 ml mammalian blood in a Vacutainer CPT tube (BD Diagnostics), then isolate PBMCs from the blood according to the instructions provided by the tube supplier.

2. -Resuspend the isolated PBMCs in cold PBS, then transfer an aliquot of this suspension to a fresh tube.
   ⚠ ***This aliquot should contain cells from no more than 400 µl of the original blood sample.***

   -Add enough PBS to the tube to make the total volume 10 ml.

3. -Centrifuge the sample for 15 minutes at 300 ×*g* at +15 to +25°C.
   -Discard the supernatant.

4. -Resuspend the appropriate number of cells in 100 ml PBS.
   -Add 100 µl Lysis/Binding Buffer (bottle 3) .
   -Mix thoroughly by vortexing.
   ⚠ ***Use only Lysis/Binding Buffer containing 1% DTT.***

5. Transfer the lysate into one well of the sample cartridge.

6. Place the sample cartridge on the reagent/sample stage, and start the 'RNA HP Cells' protocol.

Primary Cells

For sample preparation of up to 1 x 10^6 primary cells follow the procedure below.

1. Pellet cells by centrifugation for 10 minutes at 300 x *g*.

2. -Resuspend the cell pellet in 100 µl PBS.
   -Add 100 µl Lysis/Binding Buffer (bottle 3).
   -Mix thoroughly by vortexing.
   ⚠ ***Use only Lysis/Binding Buffer containing 1% DTT.***

3. Transfer the lysate into one well of the Sample Cartridge.

4. Place the sample cartridge on the reagent/sample stage, and start the 'RNA HP Cells' protocol.

Cultured Cells

For sample preparation of up to 1 ×10^6 cultured cells follow the procedure below.

1. Pellet cells by centrifugation for 10 minutes at 300 x *g*.

2. Resuspend the cell pellet in 200 µl PBS.

3. Transfer the sample into one well of the Sample Cartridge.

4. Place the sample cartridge on the reagent/sample stage, and start the 'RNA HP Cells' protocol.

Purification protocol

To perform RNA isolations with the MagNA Pure LC RNA Isolation Kit – High Performance, one of the three protocols, either the 'RNA HP Blood', 'RNA HP Cells', or 'RNA HP Blood_external_lysis' purification protocol listed below is selected on the protocol selection menu of the "Sample Ordering" screen of the MagNA Pure LC Software.

Use the following guide to select the appropriate purification protocol for your sample material. Prevent overloading of the magnetic glass particles with too much total RNA by not attempting to process more than 200 µl whole blood, and not more than 1 ×10^6blood cells and cultured cells in each well of the sample cartridge.

Protocol Name	Sample Material	Procedure
RNA HP Blood	whole blood (20-200 µl)	Samples are setup directly on the MagNA Pure LC Reagent/Sample Stage. Lysis and RNA purification are carried out automatically by the instrument.
RNA HP Blood_external_lysis		Samples are lysed manually outside of the MagNA Pure LC 2.0 Instrument. Lysates are then transferred to the Reagent/Sample Stage and RNA purification is carried out automatically by the instrument. *External lysis permits physical separation of the lysis step from the purification step. Inactivated sample is then placed into material to the MagNA Pure LC 2.0 Instrument (e.g., when using potentially infectious samples).*
RNA HP Cells	200 µl suspension of blood cells, primary cells, or cultured cells (10^3 to 10^6 cells)	Samples are setup directly on the MagNA Pure LC Reagent/Sample Stage. Lysis and RNA purification are carried out automatically by the instrument. *Blood cells require manual pre-isolation steps.*

All RNA HP purification protocols allow to set the volume of Elution Buffer in a range from 50 to 100 µl.
When using an elution volume of 50 µl, total RNA yield may be 10 – 20% lower compared to 100 µl.

General Remarks for the MagNA Pure LC RNA Isolation Protocol

- The procedure described below is designed to process 32 samples at the same time. When processing fewer samples, reduce the volumes of all solutions accordingly by following the instructions provided on the 'Stage Setup' sub tab of the MagNA Pure LC 2.0 Instruments.
- The software automatically calculates the necessary amounts of reagents and disposable plastics and guides you through the set-up.
- Please note that it is not possible to start the instrument until the interlock bar that secures the sample cartridge, reagent tubs, and reaction tips is locked in the closed position.
- When programming the optional dilution of the RNA eluate, an additional reagent tub M30 in position R8 will be required. Use either the Elution Buffer or nuclease-free water to further dilute the eluate.

High Performance RNA Isolation Protocol using the MagNA Pure LC 2.0 Instruments

	MagNA Pure LC 2.0 Instrument
Start Instrument and Software	Turn on the instrument, the MagNA Pure LC 2.0 Software starts automatically. Log in and then navigate to the '*Ordering'* sub-tab.
	Select the appropriate protocol: If you are starting with whole blood sample, then use either the *"RNA HP Blood" or RNA HP Blood_external_lysis"* protocol. If you are starting with pre-isolated WBCs or PBMCs, primary cells or cultured cells, then use the *"RNA HP Cells"* protocol.
	Follow the instructions in the software, and specify the name and number of samples. Type in sample volume, elution volume and dilution volume (if necessary). The software will calculate how much of each reagent is required.
Fill the reagent tubs	Before starting the isolation procedure, fill all reagent tubs outside the instrument with the required amount of reagents (equilibrated to +15 to +25°C).
	Fill each reagent tub with the volume listed on the '*Stage Setup*' sub-tab, then close it with a tub lid.
	⚠ ***Use only the reagent amount needed for your sample number*** ⚠ ***Close reagent tubs with the tub lids, to prevent evaporation of the reagents. Even when closed, reagent tubs are not suitable for long-term storage of reagents.*** ⚠ ***Load the exact amount of MGPs (as listed on the '****Start Information****' screen or '****Stage Setup****' sub-tab) on to the instrument. Load the MGPs just before starting the run to prevent them from sedimenting.***
Set up reagent tubs and disposables on the reagent/sample stage	Use the information of the '*Stage Setup*' sub-tab to place all disposable plastics and reagents within reagent tubs, necessary for the batch run on the reagent/sample stage.
	ⓘ *A colored "Positioning Frame" that can be placed on the reagent reservoir rack, to aid correct loading of the reagents, is available with the MagNA Pure LC Disposables Starter Set*.*
Load the samples	▪ Transfer the sample cartridge, containing the samples or lysates to the MagNA Pure LC 2.0 Instrument. ▪ Close the disposable lockbar.
Start the batch run	▪ On the '*Stage Setup'* sub-tab, confirm the correct placement of all disposable plastics and reagents, by selecting the respective button on the reagent/sample stage area. ▪ Select the '*Start'* button to start the automated total viralnucleic acids isolation procedure. The instrument will automatically dispense all reagents and process thesamples.

Storage of RNA Eluates

⚠ ***To ensure greatest possible stability of the eluted RNA, immediately proceed with RT-PCR set-up. Do not store the eluted RNA in the Storage Cartridge on Cooling Unit 1.***

For long-term storage (at least for several weeks), close the Storage Cartridge with the Cartridge Seals* and store the RNA at -60 to -80°C. It is best to store the RNA in aliquots, so the preparation will not have to be repeatedly frozen and thawed.

⚠ ***After thawing eluates, mix gently by pipetting up and down ten times before performing any downstrean steps, e.g., RT-PCR, or OD measurements. If nuceic acids are not premixed and distributed evenly/ homogenously in soluion, results may not be reproducible in subsequent assays.***

Post-Elution Steps

The MagNA Pure LC 2.0 Instrument can help set up RT-PCR reverse transcription and PCR reactions by pipetting RNA samples and master reagent mixes (for RT-PCR, RT, or PCR) into either LightCycler® Capillaries, standard PCR tubes or plates. (See the MagNA Pure LC 2.0 Operator's Manual for recommended plates.) For post-elution procedures, you can place LightCycler® Capillaries in the removable MagNA Pure LC Cooling Block, LC Centrifuge Adaptors or the MagNA Pure LC Cooling Block, LC Sample Carousel. You can program the post-elution steps either before you perform the isolation procedure or after it is finished. See the MagNA Pure LC 2.0 Operator's Manual for details how to set up a post-elution run.

3. Results

RNA integrity

RNA was purified from either 200 µl whole blood or 1 × 10^6 cultured cells (K562) using the appropriate RNA HP purification protocol. Because blood cells contain about 10 to 20-fold less RNA than cultured cells, RNA integrity was checked on agarose gels either stained with SYBR Green II (RNA from blood) or ethidiumbromide (RNA from cultured cell). All RNA samples showed good integrity as indicated by intact rRNA bands (Figures 1a, b).

Fig. 1 Agarose gel electrophoresis of RNA isolated from whole blood (1a) or K562 cells (1b)

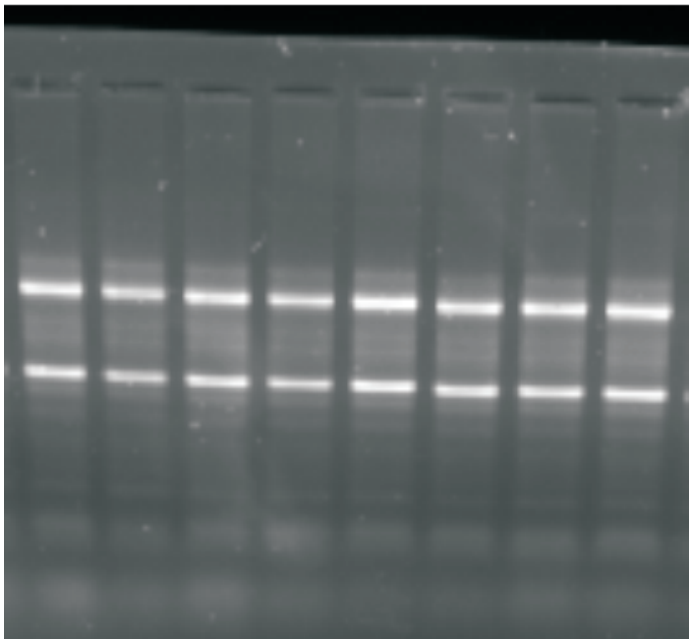

Fig. 1a: RNA from whole blood

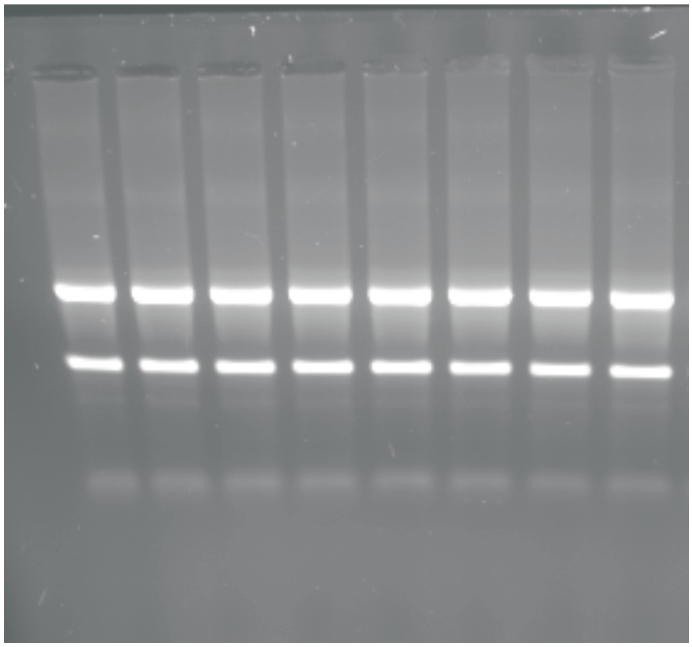

Fig. 1b: RNA from K562 cells

Yield

RNA was prepared either from different types of cultured cells or human blood of different donors. Yields were determined by OD_{260} measurement and agarose gel electrophoresis (for typical yields see Table 1).

Purity

- RNA was purified from different amounts and types of blood and cultured cells. In typical experiments, RNA had an $OD_{260/280}$ ratio of 2.0 ± 0.1, indicating highly pure RNA.
- RNA was purified from blood containing different anticoagulants (EDTA, citrate, heparin). No RT-PCR inhibition was observed in subsequent LightCycler® System analysis, indicating the absence of PCR inhibitors.
- In all experiments, a 'minus-RT' control PCR was performed for all of the samples. After 40 cycles, no signal was observed in LightCycler® System RT-PCR analysis, indicating the absence of contaminating genomic DNA.

⚠ ***Depending on the sensitivity of the RT-PCR and on the RT-PCR parameter, sometimes weak signals raising at late cycles may be observed in the minus-RT control PCR. These are usually too weak to affect the RT-PCR analysis. Roche recommends that primers be designed with respect to the exon/exon boundary of the RNA, so that genomic DNA is not amplified.***

Sample Material (amount)	RNA Yield [µg]	RNA Purity [$OD_{260/280}$]
Blood/donor 1 (200 µl)	1.15	2.09
Blood/donor 2 (200 µl)	1.10	2.09
Blood/donor 3 (200 µl)	1.21	2.08
Blood/donor 4 (200 µl)	1.32	2.07
Blood/donor 5 (200 µl)	1.38	2.08
Blood/donor 6 (200 µl)	1.40	2.09
Cultured cells/K562 (10^6)	17.50	2.00
Cultured cells/HeLa (10^6)	15.50	2.00

Reproducibility

- Intra-assay variance

RNA was purified from 16 different 200 µl aliquots from the same donor and 16 different samples of 1 × 106 K562 cells from the same source. The variations of RNA yield and purity, based on $OD_{260/OD280}$ measurements, were approx. 10%, and the variation of the LightCycler® System's Crossing Points was approx. 3%, indicating outstanding reproducibility of RNA yield within a single purification run.

- Inter-assay variance

RNA was purified in four different purification runs each time using eight samples of 1 × 10^6 cultured cells from the same source. The variations of RNA yield and purity were approx. 10%, and the variation of the LightCycler® System' s Crossing points was approx. 3%, indicating outstanding reproducibility of RNA yield in the four different purification runs.

4. Troubleshooting

Observation	Possible cause	Recommendation
Clumping of beads / problem with magnetic separation of beads	Too much sample material.	Reduce amount of sample material to the values indicated in section “Sample Material”.
	MGPs were magnetized prior to use.	Avoid contact between MGPs and magnets prior to use. Store kit appropriately.
RNA is degraded	Storage of samples was not appropriate.	Use fresh samples, whenever possible. Do not freeze whole blood before processing it. Never collect WBCs or PBMCs from a frozen blood sample
		Avoid the use of samples that have been stored extensively at +15 to +25°C.
	RNAse contamination of Reaction tips, reagent tubs, sample cartridges or reagents.	Avoid contaminating disposables and reagents with nucleases.
Poor or no RNA yield	Incorrect storage of samples	Use fresh samples, whenever possible. Avoid the use of samples that have been stored extensively at +15 to +25°C.
	Sample did not contain enough cells; improperly lysed sample.	▪ Cells: Count cells from cell culture before use. Optimal results are obtained using cell numbers up to 10^6 cells. ▪ Blood: Make sure that the blood is not clotted. Use fresh blood to which anticoagulants were added. ▪ WBCs: Preparation or lysis of WBC’s insufficient; work according instructions. See note in section “Sample Material”.
	Reagents were placed incorrectly on the reagent/sample stage.	Ensure that all reagents are in the correct positions on the Reagent/Sample Stage.
Poor RNA purity	Too much sample material or too many blood cells in the sample.	Reduce amount of sample material to the values indicated in section “Sample Material”.
	Not enough RNA for correct OD measurement or wrong pH.	Whole blood usually has a low RNA concentration making a correct OD measurement difficult. Also, the pH of the RNA preparation has an influence on the OD; if necessary spike eluate with Tris buffer (pH 7-8).
Poor PCR performance	Poor purity of RNA.	Too much sample material used for isolation. Adjust input material to the values indicated in section “Sample Material”.
	RT-PCR reagents and protocols were not optimal.	Check PCR/RT-PCR reagents and protocols with a positive RNA control.
Eluates show a slight red color	Minimal abrasion from magnetic particles	Centrifuge at low *g*-values (approx. 1,000 rpm) to remove fines. *The red color does not affect PCR or RT-PCR on LightCycler® 480 and LightCycler® Carousel-Based Instruments.*

5. Additional Information on this Product

5.1. Test Principle

MagNA Pure LC RNA Isolation Kit - High Performance is used with the MagNA Pure LC 2.0 Instrument to purify high-quality, undegraded total RNA from 1-32 samples of mammalian whole blood, WBCs, PBMCs, or cultured cells. You may lyse whole blood samples either manually outside the instrument or using automation with (on the MagNA Pure LC 2.0 Instrument). Isolated RNA can be eluted into any volume between 50 and 100 µl. Purified RNA meets the quality standards required for highly sensitive and quantitative RT-PCR analysis using LightCycler® Instruments.

Test principle

Samples are lysed by incubation using a special buffer that contains chaotropic salt. Proteinase K digestion destroys remaining proteins and nucleases. Magnetic Glass Particles (MGPs) are added and the RNA is bound to their surfaces. DNA is degraded by incubation with DNase. Unbound substances are removed by several washing steps. Purified RNA is eluted obtained using an Elution Buffer.

The principle steps of a MagNA Pure LC total RNA isolation procedure are:

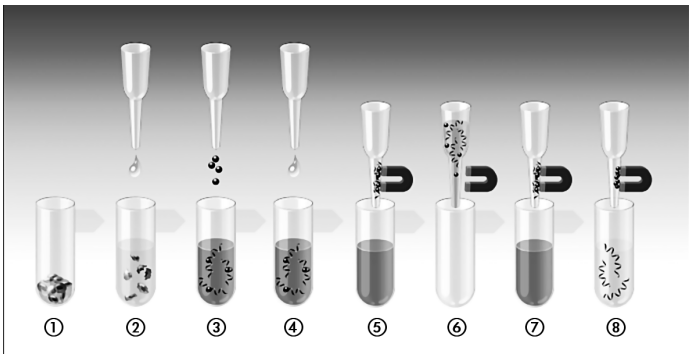

Fig. 2: The principle steps of a MagNA Pure LC total RNA isolation procedure are:

① Sample material is placed into the wells of the Sample Cartridge.

② Lysis/Binding Buffer is added to the sample, resulting in cell lysis and release of RNA. RNases are denatured.

③ RNA binds to the silica surface of the added MGPs due to the chaotropic salt conditions, isopropanol, and the high ionic strength of the Lysis/Binding Buffer.

④ Genomic DNA is removed by incubation with DNase I.

⑤ RNA is rebound to the particles by addition of isopropanol. MGPs with bound RNA are then magnetically separated from the residual lysed sample.

⑥ MGPs with bound RNA are washed repeatedly with Wash Buffer to remove unbound substances such as proteins (nucleases), cell membranes, PCR inhibitors such as heparin or hemoglobin, and to reduce the chaotropic salt conc.

⑦ MGPs with bound RNA are again magnetically separated from the Wash Buffer containing residual sample debris.

⑧ Purified RNA is eluted at +70°C from the MGPs in the wells of the Elution Cartridge. MGPs are retained in the reaction tip and discarded.

The basic steps of the MagNA Pure LC RNA Isolation Kit - High Performance isolation procedure (RNA HP Blood protocol) are as follows:

① Dispense all required reagents into the Processing Cartridge.

② Dispense Elution Buffer into the Elution Cartridge (Heating Block)

③ Add Proteinase K, mix, incubate, separate particles.

④ Mix Lysis/Binding Buffer with the samples.

⑤ Add MGP suspension, mix, incubate, separate particles.

⑥ Transfer MGPs to Wash Buffer I, mix, separate particles.

⑦ Digest with DNase, incubate, separate particles.

⑧ Add Lysis/Binding Buffer and isopropanol.

⑨ Transfer MGPs to Wash Buffer I, mix, separate particles.

⑩ Transfer MGPs to Wash Buffer II, mix, separate particles

⑪ Transfer MGPs to Wash Buffer II, mix, separate particles

⑫ Transfer MGPs into Elution Buffer (Heating Unit), mix, incubate, elute RNA in 50 to 100 µl.

⑬ Transfer eluate to the Storage Cartridge (in Cooling Unit I).

5.2. Quality Control

RNA was isolated from mammalian blood and cultured cells using the appropriate purification protocol. The quality of the purified RNA was determined using $OD_{260/280}$ readings or RT-PCR/PCR on the LightCycler® System.

6. Supplementary Information

6.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols	
Information Note: Additional information about the current topic or procedure.	
Important Note: Information critical to the success of the current procedure or use of the product.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ etc.	Steps in a procedure that must be performed in the order listed.
* (Asterisk)	The Asterisk denotes a product available from Roche Diagnostics.

6.2. Changes to previous version

- Chapter **Quality Control** has been updated.
- Editorial changes.

6.3. Ordering Information

Roche offers a large selection of reagents and systems for life science research. For a full overview of related products and manuals, please visit and bookmark our homepage lifescience.roche.com.

Product	Pack Size	Cat. No.
Consumables		
MagNA Pure LC Disposable Starter Set	1 set	03 005 488 001
Reagents, kits		
Buffers in a Box, Premixed PBS Buffer, 10x	4 l	11 666 789 001
Red Blood Cell Lysis Buffer	100 ml, 50-500 reactions, depending on sample size (1-500 µl)	11 814 389 001
1,4-Dithiothreitol	2 g	10 197 777 001
	10 g	10 708 984 001
	25 g	11 583 786 001

6.4. Trademarks

MAGNA PURE and LIGHTCYCLER are trademarks of Roche.
SYBR is a trademark of Thermo Fisher Scientific Inc..
All other product names and trademarks are the property of their respective owners.

6.5. License Disclaimer

For patent license limitations for individual products please refer to:
http://technical-support.roche.com.

6.6. Regulatory Disclaimer

For general laboratory use.

6.7. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

6.8. Contact and Support

If you have questions or experience problems with this or any Roche product for Life Science, please contact our Technical Support staff. Our scientists are committed to providing rapid and effective help.
Please also contact us if you have suggestions for enhancing Roche product performance or using our products in new or specialized ways. Such customer information has repeatedly proven invaluable to the research community worldwide.
To ask questions, solve problems, suggest enhancements or report new applications,
please visit our **Online Technical Support** Site.

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