VetMAX™ Swine Influenza A-09 Kit

Nucleic acid purification protocols

For use with Catalog Number INFAPSWINE

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Species	Isolation of nucleic acid from matrices	Test type
Porcine	Nasal swabs Organs Bronchoalveolar lavage Fetal liquid Cell culture supernatant	Individual

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⚠ **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support.**

⚠ **WARNING! POTENTIAL BIOHAZARD.** Read the biological hazard safety information at this product's page at **thermofisher.com.** Wear appropriate protective eyewear, clothing, and gloves.

Purification of nucleic acid from biological samples

Contents of this manual

This manual presents influenza virus viral RNA purification protocols compatible with the **Applied Biosystems™ VetMAX™ Swine Influenza A-09 Kit**.

Sample selection

Sample matrix	Type of analysis	Sampling equipment
Nasal swabs	Individual	Dry swab or swab with transport media
Organ	Individual	Lung, tracheal tissue
Various liquids	Individual	Bronchoalveolar lavage, fetal liquid, cell culture supernatant

Sample storage

The quality of the samples must be assured before starting the analytical process.

Nasal swabs

Storage conditions differ, depending on the type of swab used. It is advisable to use swabs with transport media for viral research.

Swab with transport media

After sampling, store as indicated below:

- If the analysis is to be performed within 24 hours of sampling: maintain at room temperature until use.
- Otherwise, maintain at +2°C to +8°C until use and for a maximum of 72 hours after sampling.

In both cases, perform the PCR with the eluate immediately after swab elution. Store the rest of the eluate below –70°C to enable subsequent viral isolation.

Dry swab

After sampling, maintain at room temperature until use and for a maximum of 4 hours after sampling.

Perform the PCR with the eluate immediately after swab elution. Store the rest of the eluate below –70°C to enable subsequent viral isolation.

Organs

After sampling, store as indicated below:

- If the analysis is to be performed within 24 hours of sampling: maintain at +2°C to +8°C until use.
- If the analysis is to be performed more than 24 hours after sampling: keep below –16°C until use.

In both cases, after use or beyond the 24-hour deadline, freeze below –16°C for storage up to 1 year or below –70°C for storage longer than 1 year.

Liquids (bronchoalveolar lavage, fetal liquid, cell culture supernatant)

Following collection, maintain at +2°C to +8°C until use and for a maximum of 72 hours after sampling. After use or after the 72-hour period, freeze below –16°C for storage up to 1 year or below –70°C for storage longer than 1 year.

Materials required but not provided

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Equipment and reagents required for the preparation of samples for analysis

- Class II microbiological safety cabinet (MSC)
- Adjustable micropipettes (range of 1 µL to 1000 µL) with DNase/RNase-free filtered tips
- Vortex or equivalent
- Centrifuge for microtubes (8000 × *g* to 15 000 × *g*)
- Laboratory mill for the mechanical grinding of the organs
- 3 mm glass beads
- 1.5 mL DNase/RNase-free microtubes
- 1X PBS buffer
- DNase/RNase-free water

Kits, reagents and equipment for extraction and purification of RNA from samples

All equipment and reagents required to prepare samples for analysis are likely to be used for this step. In addition, ensure that the following are available:

- 96–100% ethanol and 70% ethanol
- Optional: β-mercaptoethanol 14.3 M
- Manual extraction on columns of silica in individual format:
  - RNeasy™ Mini Kit + QIAshredder (QIAGEN™) or NucleoSpin™ RNA kit (MACHEREY-NAGEL)
  - Centrifuge for microtubes (8000 × *g* to 15 000 × *g*)
- Manual extraction on columns of silica in plate format:
  - NucleoSpin™ 8 RNA kit + NucleoSpin™ RNA Filter Strips or NucleoSpin™ 96 RNA kit + NucleoSpin™ RNA Filter Plate (MACHEREY-NAGEL)
  - Centrifuge for plates or a vacuum system

RNA extraction protocols

	Recommended extraction kits
Swabs, organs, liquids	RNeasy™ Mini Kit
	NucleoSpin™ RNA kit
	NucleoSpin™ 8 / 96 RNA kit

RNA extraction from swabs, organs or various liquids

EPC

The EPC to be extracted with a **VetMAX™ Swine Influenza A-09 Kit** can be replaced by an internal sentinel in the laboratory during the process of nucleic acid extraction. In this case, the sentinel is processed using the same process (preparation of the sample and extraction) as a sample of the same type (organs or liquids).

Preparation of samples before extraction

Nasal swabs

1. For a dry swab, add 2 mL of minimum essential medium (MEM) containing 100 IU/mL of penicillin and 100 µg/mL of streptomycin to the swab in its transport tube.

   NOTE: In the absence of MEM + penicillin and streptomycin, add 2 mL of physiological saline.

2. Massage the swab through the transport tube and vortex for a few seconds.
3. Viral RNA extraction will be performed on **200 µL** of swab eluate.

Put the rest of the available volume (up to 1.8 mL) in a suitable storage tube and store below –70°C (for viral isolation).

Organs

Preparation for lysis with mechanical grinding

Weigh **25 mg (±5 mg)** of the organ in a 1.5 mL microtube using a precision balance. Proceed with the extraction.

Preparation for lysis without mechanical grinding

1. Finely dissect the organ piece in a sterile Petri dish using sterile forceps and scalpel.
2. Weigh **25 mg (±5 mg)** of the previously dissected organ in a 1.5 mL microtube using a precision balance (1 mg).
3. Perform RNA extraction on the organ pieces immediately.

Liquids (bronchoalveolar lavage, fetal liquid, cell culture supernatant)

These samples are **used directly**, without preparation prior to extraction. Viral RNA extraction will be performed on **200 µL**.

Extraction with RNeasy™ Mini Kit

Note

The protocol has been validated with or without the use of β-mercaptoethanol for lysis of liquid samples. If β-mercaptoethanol is used, it must be added to the lysis buffer according to the recommendations below.

Before starting

- For organs (optional for liquid samples): Reconstitution of the "RLT+βME" buffer: Add 10 µL of β-mercaptoethanol (βME) to 1 mL of RLT buffer, under a laminar flow hood and with suitable protective clothing (including gloves, mask, gown, etc.). Only reconstitute the volume required for analysis. The prepared solution is stable for 1 month at room temperature.
- Reconstitution of the **RPE** buffer: Add the required quantity of 96–100% ethanol according to the supplier's recommendations prior to initial use.
- Prepare and identify microtubes and columns according to the number of samples to be extracted (including negative and positive controls).

Lysis of the swab eluates, liquid samples, and the 4c - EPC Influenza A component provided in the kit

1. Perform sample lysis as described below:

Components	Samples		
	Swab eluates, liquids[1]	EPC[1]	NCS
Lysis solution	350 µL of RLT (with or without ßME)	350 µL of RLT (with or without ßME)	350 µL of RLT (with or without ßME)
Test sample	200 µL of sample	200 µL of "4c - EPC Influenza A"	200 µL of MEM or physiological saline

[1] If a positive control other than 4c - EPC Influenza A is used, proceed with lysis according to the lysis method indicated in the column "Swab eluates, liquids" in the case of a liquid positive control or see the paragraph "Lysis of the organs" in case of a solid positive control.

2. Homogenize: by pipetting up and down at least 5 times or by vortexing for 15 seconds.
3. Proceed with the extraction described in the paragraph "**Viral RNA purification with RNeasy™ Mini Kit**".

Lysis of the organs

Perform sample lysis as described below:

Components	Samples	
	Organs[1]	NCS
Lysis Solution	350 µL of "RLT+ßME"	350 µL of "RLT+ßME"
Test sample	25 mg of organ	200 µL of MEM or physiological saline

[1] For lysis of the 4c - EPC Influenza A component provided in the kit, see the paragraph "Lysis of the swab eluates, liquid samples and the 4c - EPC Influenza A component provided in the kit". If a positive control other than 4c - EPC Influenza A is used, proceed with lysis according to the lysis method indicated in the column "Organs" in the case of a solid positive control or see the paragraph "Lysis of the swab eluates, liquid samples and the 4c - EPC Influenza A component provided in the kit" in case of a liquid positive control.

Lysis with mechanical grinding

1. Add 1 bead of size 3 mm - Grind for 2 minutes at 30 Hz (or equivalent program depending on the mill).
2. Briefly centrifuge. Take **supernatant**. If necessary, transfer to a QIAshredder column - Centrifuge for 2 minutes at 14 000 × *g* - Keep eluate.
3. Proceed with the extraction described in the paragraph "**Viral RNA purification with RNeasy™ Mini Kit**".

Lysis without mechanical grinding

1. **Vortex** for at least **5 minutes**.
2. Transfer to a **QIAshredder** column - Centrifuge for 2 minutes at 14 000 × *g* - Keep **350 µL of the eluate**.
3. Proceed with the extraction described in the paragraph "**Viral RNA purification with RNeasy™ Mini Kit**".

Viral RNA purification with RNeasy™ Mini Kit

1. Add **350 µL of 70% ethanol** to each tube - Homogenize by pipetting (up and down at least 10 times) or by vortexing for 15 seconds - Centrifuge rapidly before opening the tube if homogenization by vortexing is done. The sample lysate is produced.
2. Locate a mini column from the RNeasy™ Mini Kit.
3. Using a pipette, transfer **700 µL of sample lysate** to the column - Cap the column - Centrifuge for 1 minute at 8000 × *g* - Discard the collection tube - **Keep the column**.
4. Using a pipette, transfer **the remaining sample lysate** to the same column if necessary - Cap the column - Centrifuge for 1 minute at 8000 × *g* - Discard the collection tube - **Keep the column**.
5. Add **700 µL** of **RW1** buffer to each column - Cap the column - Centrifuge for 1 minute at 8000 × *g* - Discard the collection tube - **Keep the column**.
6. Add **500 µL** of **RPE** buffer (reconstituted) to each column - Cap the column - Centrifuge for 1 minute at 8000 × *g* - Discard the collection tube - **Keep the column**.
7. Add **500 µL** of **RPE** buffer (reconstituted) to each column - Cap the column.
   Optional: Centrifuge for 1 minute at 8000 × *g* - Discard the collection tube - **Keep the column**.
8. Centrifuge for 3 minutes at 14 000 × *g* (to dry the membrane) - Discard the collection tube - **Keep the column**.
9. Place the column in a **1.5 mL microtube** - Add **50 µL** of **DNase/RNase-free water** - Cap the microtube.
10. **Incubate** for **2 minutes** at room temperature.
11. Centrifuge for 1 minute at 8000 × *g* to elute - Discard the column - **Keep the microtube**.

Keep the eluates at +2°C to +8°C if the PCR is to be performed immediately, or store below –16°C.

Extraction with NucleoSpin™ RNA kit

Note

The protocol has been validated with or without the use of β-mercaptoethanol for lysis of liquid samples. If β-mercaptoethanol is used, it must be added to the lysis buffer according to the recommendations below.

Before starting

- For organs (optional for liquid samples): Reconstitution of the **"RA1+βME"** buffer: Add 10 µL of β-mercaptoethanol (βME) to 1 mL of RA1 buffer, under a laminar flow hood and with suitable protective clothing (including gloves, mask, gown, etc.). Only reconstitute the volume required for analysis. The prepared solution is stable for 1 month at room temperature.
- Reconstitution of the **RA3** buffer: Add the required quantity of 96–100% ethanol according to the supplier's recommendations prior to initial use.
- Prepare and identify microtubes and columns according to the number of samples to be extracted (including negative and positive controls).

Lysis of the swab eluates, liquid samples and the 4c - EPC Influenza A component provided in the kit

1. Perform sample lysis as described below:

Components	Samples		
	Swab eluates, liquids[1]	EPC[1]	NCS
Lysis Solution	350 µL of RA1 (with or without ßME)	350 µL of RA1 (with or without ßME)	350 µL of RA1 (with or without ßME)
Test sample	200 µL of sample	200 µL of "4c - EPC Influenza A"	200 µL of MEM or physiological saline

[1] If a positive control other than 4c - EPC Influenza A is used, proceed with lysis according to the lysis method indicated in the column "Swab eluates, liquids" in the case of a liquid positive control or see the paragraph "Lysis of the organs" in case of a solid positive control.

2. Homogenize: by pipetting up and down at least 5 times or by vortexing for 15 seconds.
3. Proceed with the extraction described in the paragraph "**Viral RNA purification with NucleoSpin™ RNA kit**".

Lysis of the organs

Perform sample lysis as described below:

Components	Samples	
	Organs[1]	NCS
Lysis solution	350 µL of "RA1+ßME"	350 µL of "RA1+ßME"
Test sample	25 mg of organ	200 µL of MEM or physiological saline

[1] For lysis of the 4c - EPC Influenza A component provided in the kit, see the paragraph "Lysis of the swab eluates, liquid samples and the 4c - EPC Influenza A component provided in the kit". If a positive control other than 4c - EPC Influenza A is used, proceed with lysis according to the lysis method indicated in the column "Organs" in the case of a solid positive control or see the paragraph "Lysis of the swab eluates, liquid samples and the 4c - EPC Influenza A component provided in the kit" in case of a liquid positive control.

Lysis with mechanical grinding

1. Add 1 bead of size 3 mm - Grind for 2 minutes at 30 Hz (or equivalent program depending on the mill).
2. Briefly centrifuge. Take **supernatant**. If necessary, transfer to a **NucleoSpin™ Filter** column - Centrifuge for 1 minute at 11 000 × *g* - Keep eluate.
3. Proceed with the extraction described in the paragraph "**Viral RNA purification with NucleoSpin™ RNA kit**".

Lysis without mechanical grinding

1. **Vortex** for at least **5 minutes**.
2. Transfer to a **NucleoSpin™ Filter** column - Centrifuge for 1 minute at 11 000 × *g* - Keep **eluate**.
3. Proceed with the extraction described in the paragraph "**Viral RNA purification with NucleoSpin™ RNA kit**".

Viral RNA purification with NucleoSpin™ RNA-kit

1. Add **350 µL of 70% ethanol** to each tube - Homogenize by pipetting (up and down at least 10 times) or by vortexing for 15 seconds - Centrifuge rapidly before opening the tube if homogenization by vortexing is done - The **sample lysate** is produced.
2. Identify a mini column from the NucleoSpin™ RNA Kit.
3. Using a pipette, transfer **700 µL of sample lysate** to the column - Cap the column - Centrifuge for 30 seconds at 11 000 × *g* - Discard the collection tube - **Keep the column**.
4. Using a pipette, transfer **the remaining sample lysate** to the same column if necessary - Cap the column - Centrifuge for 30 seconds at 11 000 × *g* - Discard the collection tube - **Keep the column**.
5. Add **200 µL** of **RA2** buffer to each column - Cap the column - Centrifuge for 30 seconds at 11 000 × *g* - Discard the collection tube - **Keep the column**.
6. Add **600 µL** of **RA3** buffer (reconstituted) to each column - Cap the column - Centrifuge for 30 seconds at 11 000 × *g* - Discard the collection tube - **Keep the column**.
7. Add **250 µL** of **RA3** buffer (reconstituted) to each column - Cap the column.
   Optional: Centrifuge for 30 seconds at 11 000 × *g* - Discard the collection tube - **Keep the column**.
8. Centrifuge for 2 minutes at 11 000 × *g* (to dry the membrane) - Discard the collection tube - **Keep the column**.
9. Place the column in a **1.5 mL microtube** - Add **60 µL** of **DNase/RNase-free water** - Cap the microtube.
10. **Incubate** for **2 minutes** at room temperature.
11. Centrifuge for 1 minute at 11 000 × *g* to elute - Discard the column - **Keep the microtube**.

Keep the eluates at +2°C to +8°C if the PCR is to be performed immediately, or store below –16°C.

Extraction with NucleoSpin™ 8 / 96 RNA kits

Note

Extractions with the NucleoSpin™ 8 RNA and NucleoSpin™ 96 RNA kits are similar, only the format of columns differs: in strips of 8 columns or a plate of 96 columns.

Before starting

- Reconstitution of the "**RA1+βME"** buffer: Add 10 µL of β-mercaptoethanol (βME) to 1 mL of RA1 buffer, under a laminar flow hood and with suitable protective clothing (including gloves, mask, gown, etc.). Only reconstitute the volume required for analysis. The prepared solution is stable for 1 month at room temperature.
- Reconstitution of the **RA3** buffer: Add the required quantity of 96–100% ethanol according to the supplier's recommendations prior to initial use.
- Reconstitution of the **RA4** buffer: Add the required quantity of 96–100% ethanol according to the supplier's recommendations prior to initial use.
- Prepare and identify microtubes and columns according to the number of samples to be extracted (including negative and positive controls).

Lysis of the swab eluates, liquid samples and the 4c - EPC Influenza A component provided in the kit

1. Perform sample lysis as described below:

Components	Samples		
	Swab eluates, liquids[1]	EPC[1]	NCS
Lysis solution	300 µL of "RA1+ßME"	300 µL of "RA1+ßME"	300 µL of "RA1+ßME"
Test sample	200 µL of sample	200 µL of "4c - EPC Influenza A"	200 µL of MEM or physiological saline

[1] If a positive control other than 4c - EPC Influenza A is used, proceed with lysis according to the lysis method indicated in the column "Swab eluates, liquids" in the case of a liquid positive control or see the paragraph "Lysis of the organs" in case of a solid positive control.

2. Homogenize: by pipetting up and down at least 5 times or by vortexing for 15 seconds.
3. Proceed with the extraction described in the paragraph "**Viral RNA purification with NucleoSpin™ 8 / 96 RNA kits**".

Lysis of the organs

Perform sample lysis as described below:

Components	Samples	
	Organs[1]	NCS
Lysis solution	300 µL of "RA1+ßME"	300 µL of "RA1+ßME"
Test sample	25 mg of organ	200 µL of MEM or physiological saline

[1] For lysis of the 4c - EPC Influenza A component provided in the kit, see the paragraph "Lysis of the swab eluates, liquid samples and the 4c - EPC Influenza A component provided in the kit". If a positive control other than 4c - EPC Influenza A is used, proceed with lysis according to the lysis method indicated in the column "Organs" in the case of a solid positive control or see the paragraph "Lysis of the swab eluates, liquid samples and the 4c - EPC Influenza A component provided in the kit" in case of a liquid positive control.

Lysis with mechanical grinding

1. Add 1 bead of size 3 mm - Grind for 2 minutes at 30 Hz (or equivalent program depending on the mill).
2. Briefly centrifuge. Take **supernatant**. If necessary, transfer to **NucleoSpin™ RNA Filter Strips** or **NucleoSpin™ RNA Filter Plate** filter columns - Centrifuge for 1 minute at 6000 × *g* - Keep the eluate.
3. Proceed with the extraction described in the paragraph "**Viral RNA purification with NucleoSpin™ 8 / 96 RNA kits**".

Lysis without mechanical grinding

1. **Vortex** for at least **5 minutes**.
2. Transfer to **NucleoSpin™ RNA Filter Strips** or **NucleoSpin™ RNA Filter Plate** filter columns - Centrifuge for 1 minute at 6000 × *g* - Keep **the eluate**.
3. Proceed with the extraction described in the paragraph "**Viral RNA purification with NucleoSpin™ 8 / 96 RNA kits**".

Viral RNA purification with NucleoSpin™ 8 / 96 RNA kits

1. Add **300 µL** of **RA4** buffer (reconstituted) to each tube - Homogenize by pipetting (up and down at least 10 times) - **Do not vortex** - The **sample lysate** is produced.
2. Identify columns from the NucleoSpin™ 8 / 96 RNA Kit, in strip format ("NucleoSpin RNA Binding Strips") or plate format ("NucleoSpin RNA Binding Plate") - Place them on a "MN Square-Well Block".
3. Using a pipette, transfer **all of the sample lysate** to the columns - Cover with adhesive film - Centrifuge for 2 minutes at 6000 × *g* - **Transfer the columns** to another "MN Square-Well Block" or to unused wells.
4. Add **500 µL** of **RA3** buffer (reconstituted) to each column - Cover with adhesive film - Centrifuge for 2 minutes at 6000 × *g* - **Transfer the columns** to another "MN Square-Well Block" or to unused wells.
5. Add **500 µL** of **RA2** buffer to each column - Cover with adhesive film - Centrifuge for 2 minutes at 6000 × *g* - **Transfer the columns** to another "MN Square-Well Block" or to unused wells.
6. Add **800 µL** of **RA3** buffer (reconstituted) to each column - Cover with adhesive film - Centrifuge for 2 minutes at 6000 × *g* - Transfer the columns to another "MN Square-Well Block" or to unused wells.
7. Add **500 µL** of **RA4** buffer (reconstituted) to each column - Cover with adhesive film - Centrifuge for 10 minutes at 6000 × *g* - **Transfer the columns** to another "MN Square-Well Block" or to unused wells.
8. **Transfer the columns** to elution strips or an elution plate - Add **75 µL of DNase/RNase-free water** - Cap the columns.
9. **Incubate** for **2 minutes** at room temperature.
10. Centrifuge for 3 minutes at 6000 × *g* to elute - Discard the columns - **Cap and keep the elution tubes**.

Keep the eluted samples at +2°C to +8°C if the amplification is to be performed immediately, or store the RNA below –16°C.

Extraction with NucleoSpin™ 8 RNA kit in a vacuum system

Note

This protocol is only validated for the extraction of liquid samples.

The vacuum system consists of a vacuum chamber equipped with a container fastened by 2 adapters, and a disposable, 96-well funnel plate (MN Wash Plate) to be placed in the container. The columns in strips are placed on a metal support to be placed in the vacuum chamber.

The vacuum chamber is connected to an electric pump by a pipe with a valve that remains closed to protect the samples from contamination. The vacuum is applied by switching on the vacuum pump, then adjusting the vacuum by turning the wheel of the pressure gauge, and finally by opening the valve to release the pressure. When the liquid has passed through the membrane, the valve is to be closed before switching off the pump. To release the pressure, open the valve and then close it again.

Before starting

- Reconstitution of the "**RA1+βME"** buffer: Add 10 µL of β-mercaptoethanol (βME) to 1 mL of RA1 buffer, under a laminar flow hood and with suitable protective clothing (including gloves, mask, gown, etc.). Only reconstitute the volume required for analysis. The prepared solution is stable for 1 month at room temperature.
- Reconstitution of the **RA3** buffer: Add the required quantity of 96–100% ethanol according to the supplier's recommendations prior to initial use.
- Reconstitution of the **RA4** buffer: Add the required quantity of 96–100% ethanol according to the supplier's recommendations prior to initial use.
- Prepare and identify microtubes and columns according to the number of samples to be extracted (including negative and positive controls).
- If the extraction has less than 6 strips of columns, it is necessary to supplement the system with special strips with rubber columns so as to apply an effective vacuum.

Protocol

1. Perform sample lysis as described below:

	Swab eluates, liquids	**EPC**	**NCS**
Lysis solution	300 µL of "RA1+ßME"	300 µL of "RA1+ßME"	300 µL of "RA1+ßME"
Test sample	200 µL of sample	200 µL of "4c - EPC Influenza A"	200 µL of MEM or physiological saline

2. Add **300 µL** of **RA4** buffer (reconstituted) to each tube - Homogenize by pipetting (up and down at least 10 times) - **Do not vortex** - The **sample lysate** is produced.
3. Identify columns from the NucleoSpin™ 8 RNA kit (blue columns: "NucleoSpin RNA Binding Strips") - Place them on a "MN Square-Well Block".
4. Using a pipette, transfer **700 µL of sample lysate** to the columns - Place in the vacuum chamber - Apply a **low vacuum (50 mbar)** to pass the sample drop by drop.
5. Add **500 µL** of **RA2** buffer to each column - Apply a **vacuum of 200 to 400 mbar** while the sample is passing (**about 1 min**).
6. Add **800 µL** of **RA3** buffer (reconstituted) to each column - Apply a **vacuum of 200 to 400 mbar** while the sample is passing (**about 1 min**).
7. Add **500 µL** of **RA4** buffer (reconstituted) to each column - Apply a **vacuum of 200 to 400 mbar** while the sample is passing (**about 1 min**).
8. Place the strips of columns on the metal rack "Column Holder C", which is fixed on a "MN Square-Well Block" plate (by separating them in two to balance the centrifuge pods) - **Dry the columns by centrifuging** at 6000 × *g* for 10 minutes.
9. Transfer the rack "Column Holder C" containing the strips of columns to elution strips or an elution plate - Add **75 µL of DNase/RNase-free water** - Cap.
10. **Incubate** for **2 minutes** at room temperature.
11. Centrifuge for 3 minutes at 6000 × *g* to elute - Discard the columns - **Cap and keep the elution tubes**.

Keep the eluted samples at +2°C to +8°C if the amplification is to be performed immediately, or store the RNA below –16°C.

Documentation and support

Customer and technical support

Technical support: visit **thermofisher.com/askaquestion**

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Order and web support
- User guides, manuals, and protocols
- Certificates of Analysis
- Safety Data Sheets (SDSs; also known as MSDSs)
  NOTE: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

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The information in this guide is subject to change without notice.

Revision history of Pub. No. MAN0008787 (English)

Revision	Date	Description
A.0	14 July 2017	• Addition of ß-mercaptoethanol to the lysis buffers is optional only in the case of liquid samples. • Modification of the volume of lysis supernatant used in the extraction steps. • Updated to the current document template, with associated updates to the warranty, trademarks, and logos.
1.0	30 September 2013	Baseline for revision history

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