Nexxo-Prep RNA mini

RNA extraction, by spin-column system, for the isolation of up to 100 µg total RNA from cell cultures (max. 1.10^7 cells), tissues (max. 20 mg), paraffin-embedded tissues or blood (max. 1.50 ml).

Eluted RNA is ready for down-stream applications and can directly be used for Northern blots, dot blots, RT-PCR, DDRT-PCR, construction of cDNA libraries or can be stored for future use.

Note: this kit has not been validated for the extraction of viral RNA and has not been tested for the isolation from serum or plasma.

I. Kit components

	10 preps	50 preps	250 preps
Tampon d'élution KL (Elution Buffer KL)	2 ml	15 ml	30 ml
Tampon de lyse LT (Lysis Buffer LT)	10 ml	50 ml	250 ml
Tampon R1 (Buffer R1)	30 ml	30 ml	4 x 30 ml
Solution de lavage M1 (Wash Solution M1)	15 ml (ready-to-use)	20 ml (final volume: 40 ml)	80 ml (final volume: 160 ml)
Solution de lavage M2 (Wash Solution M2)	15 ml (ready-to-use)	2 x 12 ml (final volume: 2 x 60 ml)	2 x 40 ml (final volume: 2 x 200 ml)
Billes Z1 (Beads Z1)	1	1	5
Billes Z2 (Beads Z2)	1	1	5
Kit Filtres ARN (RNA Filter Set)	10	50	5 x 50
Filtres ADN (DNA Filter)	10	50	5 x 50
Tubes receveurs 2,0 ml (2,0 ml Receiver Tubes)	20	2 x 50	10 x 50
Tubes receveurs GS (Receiver Tubes GS)	10	50	5 x 50
Tubes d'élution (Elution Tubes)	10	50	5 x 50
User guide	1	1	1
Art. No.	2034.10	2034.50	2034.250

Required material and equipment not included in this kit

- 1M DTT
- Ethanol >96 %
- Ethanol >70 %
- Octane/xylene, proteinase K, TE buffer (only for isolation from FFPE tissue material)
- Tubes for erythrocytes lysis (e.g. 15 ml Falcon)
- Microcentrifuge (min 11000 x g)
- Refrigerated centrifuge (only for isolation from blood samples)
- Pipettes with corresponding tips (RNase-free, sterile)
- Disposable gloves
- Bottle (1 liter)

Some components are delivered in concentrated form and have to be diluted appropriately (see chapter « Reagents and buffer solutions preparation », page 2).

II. Storage and stability

All kit components, except diluted **Buffer R1**, should be stored at room temperature (15-30 °C).

- Store diluted **Buffer R1** at +4 °C.
- This kit needs a 1M DTT solution (not included). DTT is very instable in solution. It is recommended to store 1M DTT solution at -20 °C.

  Note: do not repeat freeze-thaw cycles of 1M DTT solution. Make aliquots if necessary.

  By following these recommendation, 1M DTT is stable for 12 months.

Note: all kit components are stable for at least 12 months.

Not: 1M DTT can be replaced by 1M β-mercaptoethanol.

Ethanol is a volatile compound. Keep **Wash Solution M1** and **Wash Solution M2** tightly closed.

Bring all components to room temperature (15-30°C) and check solutions for absence of precipitates before use. Redisolve precipitates by heating (< 30 °C).

III. Reagents and buffer solutions preparation

Prepare the buffers and solutions with RNase-free ddH_2O (DEPC treated)

1. Kit 10 extractions:

- Transfer the concentrated **Buffer R1** (30 ml) into a bottle containing 970 ml of H_2O. Annotate the bottle (**"Diluted Buffer R1" + "Date")** and store at +4 °C.

Note: this kit needs a 1M DTT solution (not included). DTT is very instable in solution. It is preferable to prepare a new solution for each use.
It is however possible to prepare a stock of 1M DTT aliquots, and store them as described in chapter "Storage and stability" page 2.

Note: 1M DTT can be replaced by 1M β-mercaptoethanol.

*Note: in the 10 extractions kit **Wash Solution M1** and **Wash Solution M2** are ready-to-use.*

2. Kit 50 extractions:

- Transfer the concentrated **Buffer R1** (30 ml) into a bottle containing 970 ml of H_2O. Annotate the bottle (**"Diluted Buffer R1" + "Date")** and store at +4 °C.
- Add 20 ml of >96 % ethanol to the **Wash Solution M1**. Mix and store the bottle tightly closed.
- Add 48 ml of >96 % ethanol to each **Wash Solution M2**. Mix and store the bottle tightly closed.

Note: this kit needs a 1M DTT solution (not included). DTT is very instable in solution. It is preferable to prepare a new solution for each use.
It is however possible to prepare a stock of 1M DTT aliquots, and store them as described in chapter "Storage and stability" page 2.

Note: 1M DTT can be replaced by 1M β-mercaptoethanol.

3. Kit 250 extractions:

- Transfer each concentrated **Buffer R1** (30 ml) into a bottle containing 970 ml of H_2O. Annotate the 4 bottles (**"Diluted Buffer R1" + "Date")** and store at +4 °C.
- Add 80 ml of >96 % ethanol to the **Wash Solution M1**. Mix and store the bottle tightly closed.
- Add 160 ml of >96 % ethanol to each **Wash Solution M2**. Mix and store the bottle tightly closed.

Note: this kit needs a 1M DTT solution (not included). DTT is very instable in solution. It is preferable to prepare a new solution for each use.
It is however possible to prepare a stock of 1M DTT aliquots, and store them as described in chapter "Storage and stability" page 2.

Note: 1M DTT can be replaced by 1M β-mercaptoethanol.

IV. Protocol 1: RNA isolation from cell culture (up to 1.10^7 cells)

Before starting

- Ensure that reagents/buffers preparation has been done (see chapter "Reagents and buffer solutions preparation", page 2).
- The last step needs to place the elution tube in ice. Prepare the ice in due time.

Note: always use RNase-free consumables.

Note: 1M DTT can be replaced by 1M β-mercaptoethanol.

- Supplement the required amount of **Lysis Buffer LT** with 1M DTT at 1 % of final volume. (see step 1c or 2)

e.g. : 693 µl **Lysis Buffer LT** + 7 µl DTT 1M = 700 µl **Lysis Buffer LT** supplemented with DTT

Note: the mix Lysis buffer + DTT is instable over time, only prepare the required amounts for the protocol.

*Note: **Lysis Buffer LT** contents DNA binding particles. Shake gently before use, to re-suspend the particles. Wait until foam disappearance.*

Note: To prevent contamination, use new pipet tip for each pipetting step.

Depending on sample characteristics start with step 1a, 1b or 1c:

- 1a: for cell suspensions.
- 1b: for monolayer cells, excepted monolayers on 6 – 96 well plates, on dishes ≤ ∅ 35 mm or on flasks ≤ 12.5 cm^2. (max. 1 x 10^7 cells)
- 1c: for monolayer cells on 6 – 96 well plates or on dishes ≤ ∅ 35 mm or on flasks ≤ 12.5 cm^2.

1a	**Cell harvesting**, from a cell suspension
	• Centrifuge 5 min at 240 x g, the cell culture containing up to 1.10^7 cells. • Discard carefully (without disturbing the pellet) the supernatant and the whole culture media • Proceed with step 2 **«Cell lysis»**

1b	**Cell harvesting,** from a monolayer cell culture
	• Detach adherent cells by trypsinization • Transfer the cells into a centrifuge tube • Centrifuge 5 min at 240 x g • Discard carefully (without disturbing the pellet) the whole supernatant • Proceed with step 2 **«Cell lysis»**

Steps 1c to 7 →

1c	**Cell harvesting <u>and cell lysis</u>,** from a monolayer cell culture
	• Discard the whole cell culture media • Add directly the required amount(*) of DTT supplemented **Lysis Buffer LT** (shake gently before use) to the cell monolayer
	(*) Monolayer on 12, 24 and 96 well plates: 350 µl of DTT supplemented **Lysis Buffer LT**. Monolayer on 6 well plates, on ∅ 35 mm dishes or 12.5 cm^2 flasks: 700 µl of DTT supplemented **Lysis Buffer LT**.
	• Collect the cell lysate with a cell scraper • Transfer, with a pipette, the lysate into a reaction tube (not supplied) • Mix entirely by pipetting (any pellets or cell clumps should remain) • Proceed with step 3 « **DNA elimination** »

2	**Cell lysis**
	• Detach the cell pellet by flicking the tube • Add the required amount(*) of DTT supplemented **Lysis Buffer LT** (shake gently before use)
	(*) Pellet with less than 5 x 10^6 cells : 350 µl of DTT supplemented **Lysis Buffer LT**. Pellet with 5 x 10^6 to 1 x 10^7 cells: 700 µl of DTT supplemented **Lysis Buffer LT**.
	• Mix entirely by pipetting (any pellets or cell clumps should remain)

Note: passing the lysate through a gauge 20 needle improves the RNA extraction yield. (Shearing occurs and DNA breaks down).

3	**DNA elimination**
	• Insert a **DNA Filter** into a **2,0 ml Receiver Tube** (with lid) • Transfer the lysate from step 1c or 2 (as the case may be) into the **DNA Filter** • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g • Discard the **DNA Filter** ***Note: keep the DNA Filter if the DNA extraction is also intended.***

4	**RNA adsorption to the RNA Filter**
	• Add the required amount(*) of ethanol (70 %) to the flow-through
	(*) Less than 5 x 10^6 cells: 250 µl of ethanol (70 %). From 5 x 10^6 to 1 x 10^7 cells: 500 µl of ethanol (70 %).
	• Mix entirely by pipetting • Transfer the mixture into a **RNA Filter set** (RNA filter in his receiver tube GS) • Incubate 1 min. at room temperature • Centrifuge 1 min. at 11000 x g • Discard the flow-through and put the **RNA Filter** back into the **Receiver Tube GS** *Note: if the samples volume exceeds 700 µl, centrifuge the flow-through + ethanol mixture by successive steps.*

Steps 5 to 7 →

5	**RNA washing, step I**
	• Add 600 µl of **Wash Solution M1** to the **RNA Filter** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and the Receiver tube • Insert the **RNA Filter** into a new **Receiver Tube GS**

6	**RNA washing, step II**
	• Add 700 µl of **Wash Solution M2** to the **RNA Filter** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and put the **RNA Filter** back into the **Receiver Tube GS** • Repeat 1 X the washing-centrifugation step • Discard the flow-through and put the **RNA Filter** back into the **Receiver Tube GS** • Centrifuge 4 min. at max. speed, to remove remaining ethanol

7	**Elution of total RNA**
	• Insert the **RNA Filter** into a RNase-free **Elution Tube** • Add 40 – 100 µl of **Elution Buffer KL** (depending on desired yield and concentration) • Incubate 2 min. at room temperature • Centrifuge 1 min. at 11000 x g • Discard the **RNA Filter** and place immediately the **Elution Tube** with eluted RNA on ice

Eluted RNA is ready for down-stream applications and can directly be used for Northern blots, dot blots, RT-PCR, DDRT-PCR, construction of cDNA libraries, etc., or can be stored at -80 °C for several weeks.

Note: RNA elution can also be achieved with RNase-free ddH_2O.

V. Protocol 2: RNA extraction from whole blood (0.5 – 1.5 ml, <1.10^7 leukocytes)

Before starting

- Ensure that reagents/buffers preparation has been done (see chapter "Reagents and buffer solutions preparation", page 2).
- The first and the last step needs to place the tube in ice. Prepare the ice in due time.
- The first step needs **Diluted Buffer R1** refrigerated at 4 °C
- The first step needs a refrigerated centrifuge (4 °C)

Note: always use RNase-free consumables.

Note: 1M DTT can be replaced by 1M β-mercaptoethanol.

Note: To prevent contamination, use new pipet tip for each pipetting step.

- Supplement the required amount of **Lysis Buffer LT** with 1M DTT at 1 % of final volume. (see step 2)

e.g. : 693 µl **Lysis Buffer LT** + 7 µl DTT 1M = 700 µl **Lysis Buffer LT** supplemented with DTT

Note: the mix Lysis buffer + DTT is instable over time, only prepare the required amounts for the protocol.

*Note: **Lysis Buffer LT** contents DNA binding particles. Shake gently before use, to re-suspend the particles. Wait until foam disappearance.*

1	**Leukocytes concentration** • Homogenize the sample by inverting carefully (min. 15 to 20 inversions) • Transfer 0.5 - 1.5 ml of the sample into a 15 ml tube (not supplied) and add 10 ml of refrigerated (4 °C) **Diluted Buffer R1** • Mix briefly, but entirely, by inverting • Incubate 15 - 20 min. in ice and mix briefly, during incubation, by inverting 2 times *Note: for fresh blood (< 3 hours) increase the incubation time to 45 min.* • Centrifuge 5 min., at **4 °C**, at 960 x g • Remove delicately the supernatant (retain only the pellet) • Add 5 ml of refrigerated (4 °C) **Diluted Buffer R1** to the pellet • Mix by snipping the tube with the finger • Centrifuge 5 min., at **4 °C**, at 960 x g • Remove the whole supernatant (red interface included), and retain only the small white pellet

2	**Nucleic acids extraction** • Add 900 µl of DTT supplemented **Lysis Buffer LT** (shake gently before use) • Mix by pipetting until pellet is entirely resuspended (any pellets or cell clumps should remain) *Note: gelatinous looking particles from DNA/ **Lysis Buffer LT** interaction, are not to dissolve.*

3	**DNA elimination** • Transfer the solution from step 2 into a 2.0 ml receiver tube • Vortex 10 sec. • Incubate 5 min. at room temperature and vortex 3 – 5 times during incubation • Centrifuge 1 min. at 11000 x g • Transfer the supernatant into a new 2.0 ml receiver tube. Do not transfer the pellet, gelatinous parts or mineral particles • Add 750 µl of >96 % ethanol to the tube containing the supernatant Au tube • Mix by pipetting

4	**RNA adsorption to the RNA Filter, <u>by successive steps</u>** • Transfer the first 800 µl of the solution from the previous step (supernatant + ethanol), into the center of a **RNA Filter** (filter inserted in new tube) • Incubate 1 min. at room temperature • Centrifuge 1 min. at 11000 x g • Discard the flow-through and put the **RNA Filter** back into the tube • Transfer the leftover of the solution from step 3 (supernatant + ethanol) into the center of the **RNA Filter** • Incubate 1 min. at room temperature • Centrifuge 1 min. at 11000 x g • Discard the flow-through and put the **RNA Filter** back into the tube

Steps 5 to 7 →

5	**RNA washing, step I** • Add 600 µl of **Wash Solution M1** to the **RNA Filter** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and the Receiver tube • Insert the **RNA Filter** into a new **Receiver Tube GS**

6	**RNA washing, step II** • Add 700 µl of **Wash Solution M2** to the **RNA Filter** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and put the **RNA Filter** back into the **Receiver Tube GS** • Repeat 1 X the washing-centrifugation step • Discard the flow-through and put the **RNA Filter** back into the **Receiver Tube GS** • Centrifuge 4 min. at max. speed, to remove remaining ethanol

7	**Elution of total RNA** • Insert the **RNA Filter** into a RNase-free **Elution Tube** • Add 30 – 60 µl of **Elution Buffer KL** (depending on desired yield and concentration) • Incubate 2 min. at room temperature • Centrifuge 1 min. at 11000 x g • Discard the **RNA Filter** and place immediately the **Elution Tube** with eluted RNA on ice

Eluted RNA is ready for down-stream applications and can directly be used for Northern blots, dot blots, RT-PCR, DDRT-PCR, construction of cDNA libraries, etc., or can be stored at -80 °C for several weeks.

Note: RNA elution can also be achieved with RNase-free ddH_2O.

*Note: for RNA extraction from a buffy coat pellet obtained by centrifugation, start directly from step 2 "**Nucleic acids extraction**" (pellet must be entirely free from supernatant).*

VI. Protocol 3: RNA extraction from up to 20 mg tissue

Before starting

- Ensure that reagents/buffers preparation has been done (see chapter "Reagents and buffer solutions preparation", page 2).
- The last step needs to place the elution tube in ice. Prepare the ice in due time.

Note: always use RNase-free consumables.

Note: 1M DTT can be replaced by 1M β-mercaptoethanol.

- Supplement the required amount of **Lysis Buffer LT** with 1M DTT at 1 % of final volume. (see step 1a or 1b)

e.g. : 693 µl **Lysis Buffer LT** + 7 µl DTT 1M = 700 µl **Lysis Buffer LT** supplemented with DTT

Note: the mix Lysis buffer + DTT is instable over time, only prepare the required amounts for the protocol.

*Note: **Lysis Buffer LT** contents DNA binding particles. Shake gently before use, to re-suspend the particles. Wait until foam disappearance.*

Note: To prevent contamination, use new pipet tip for each pipetting step.

Depending on sample characteristics, manual (1a) or automated (1b) grinding approach is more appropriated

1a	**Automated sample grinding**
	• Transfer the sample in a suitable container (not supplied) adapted for grinding with a vortex, homogenizer, bead mills …
	• Add 6 **Beads Z1** and 3 **Beads Z2**
	• Add 600 µl of DTT supplemented **Lysis Buffer LT** (shake gently before use)
	• Grind and homogenise the sample
	• Transfer the sample into a 2.0 ml receiver tube
	• Proceed with step 2 "**DNA elimination**"

1b	**Manual sample grinding**
	• Grinding of the starting material by using a pestle and liquid nitrogen
	• Transfer the resulting powder in a 2.0 ml receiver tube *Note : do not thaw the sample*
	• Add 600 µl of DTT supplemented **Lysis Buffer LT** (shake gently before use)
	• Incubate under continuous shaking at room temperature until having a homogeneous lysate

Note: passing the lysate through a gauge 20 needle improves the RNA extraction yield. (Shearing occurs and DNA breaks down).

Steps 2 to 6 →

2	**DNA elimination** (and beads removal where necessary) • Centrifuge 2 min. at max. speed • Transfer carefully approx. 500 µl of the supernatant into a new 2.0 ml collection tube (not supplied) • Add 330 µl of >96 % ethanol into the new 2.0 ml collection tube • Mix entirely by pipetting

3	**RNA adsorption to the RNA Filter** • Transfer the whole solution from the collection tube of the previous step into the center of the **RNA Filter** from a **RNA Filter Set** (filter inserted in a tube) • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g • Discard the flow-through and put the **RNA Filter** back into the tube

4	**RNA washing, step I** • Add 600 µl of **Wash Solution M1** to the **RNA Filter** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and the Receiver tube • Insert the **RNA Filter** into a new **Receiver Tube GS**

5	**RNA washing, step II** • Add 700 µl of **Wash Solution M2** to the **RNA Filter** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and put the **RNA Filter** back into the **Receiver Tube GS** • Repeat 1 X the washing-centrifugation step • Discard the flow-through and put the **RNA Filter** back into the **Receiver Tube GS** • Centrifuge 4 min. at max. speed, to remove remaining ethanol

6	**Elution of total RNA** • Insert the **RNA Filter** into a RNase-free **Elution Tube** • Add 30 – 60 µl of **Elution Buffer KL** (depending on desired yield and concentration) • Incubate 2 min. at room temperature • Centrifuge 1 min. at 11000 x g • Discard the **RNA Filter** and place immediately the **Elution Tube** with eluted RNA on ice

Eluted RNA is ready for down-stream applications and can directly be used for Northern blots, dot blots, RT-PCR, DDRT-PCR, construction of cDNA libraries, etc., or can be stored at -80 °C for several weeks.

Note: RNA elution can also be achieved with RNase-free ddH_2O.

VII. Protocol 4: RNA extraction from formalin-fixed, paraffin-embedded tissues (FFPE)

Before starting

- Ensure that reagents/buffers preparation has been done (see chapter "Reagents and buffer solutions preparation", page 2).

  Note: always use RNase-free consumables.

- Prepare in due time octane or xylene (not supplied).
- Prepare in due time the proteinase K (40 mg/ml) (not supplied).
- Prepare in due time 1 mM DTT (not supplied).
- Prepare in due time the RNase-free TE buffer (not supplied).
- The last step needs to place the elution tube in ice. Prepare the ice in due time.

Note: To prevent contamination, use new pipet tip for each pipetting step.

- Supplement the required amount of **Lysis Buffer LT** with 1M DTT at 1 % of final volume. (see step 1a or 1b of protocol 3, page 10)

e.g. : 693 µl **Lysis Buffer LT** + 7 µl DTT 1M = 700 µl **Lysis Buffer LT** supplemented with DTT

Note: the mix Lysis buffer + DTT is instable over time, only prepare the required amounts for the protocol.

*Note: **Lysis Buffer LT** contents DNA binding particles. Shake gently before use, to re-suspend the particles. Wait until foam disappearance.*

1	**Deparaffinization** • Transfer the sample into a 1.5 ml reaction tube (not supplied) • Add 0.5 ml octane or xylene • Vortex gently until paraffin is dissolved • Centrifuge 2 min. at max. speed • Remove delicately the supernatant (retain only the pellet) *Note: if it remains some paraffin, centrifuge again 2 min. at max. speed and remove delicately the supernatant.* • Wash the pellet with >96 % ethanol, then dry it • Centrifuge briefly • Remove ethanol with a pipette • Incubate the open tube at 52 °C to remove the remaining ethanol

2	**Preliminary cell lysis** • Add 10 µl of proteinase K (40 mg/ml), 90 µl of RNase-free TE buffer and DTT at final concentration 10 mM (approx. 1µl of 1M DTT) *Note: mechanical grinding is recommended before or during the lysis.* • Mix entirely by pipetting • Incubate 10 min. at 48 °C • Incubate 10 min. under continuous shaking at 80 °C

Proceed with step 1 of protocol 3 "RNA extraction from up to 20 mg tissue" (page 10), with the whole sample.

VIII. Protocol 5: RNA isolation from up to 20 mg lung, kidney or spleen

Before starting

- Ensure that reagents/buffers preparation has been done (see chapter "Reagents and buffer solutions preparation", page 2).
- The last step needs to place the elution tube in ice. Prepare the ice in due time.

Note: always use RNase-free consumables.

Note: 1M DTT can be replaced by 1M β-mercaptoethanol.

- Supplement the required amount of **Lysis Buffer LT** with 1M DTT at 1 % of final volume. (see step 1a or 1b)

e.g. : 693 µl **Lysis Buffer LT** + 7 µl DTT 1M = 700 µl **Lysis Buffer LT** supplemented with DTT

Note: the mix Lysis buffer + DTT is instable over time, only prepare the required amounts for the protocol.

*Note: **Lysis Buffer LT** contents DNA binding particles. Shake gently before use, to re-suspend the particles. Wait until foam disappearance.*

Note: To prevent contamination, use new pipet tip for each pipetting step.

Depending on sample characteristics, manual (1a) or automated (1b) grinding approach is more appropriated

1a	**Automated sample grinding**
	• Transfer the sample in a suitable container (not supplied) adapted for grinding with a vortex, homogenizer, bead mills … • Add 6 **Beads Z1** and 3 **Beads Z2** • Add 900 µl of DTT supplemented **Lysis Buffer LT** (shake gently before use) • Grind and homogenise the sample • Transfer the sample into a 2.0 ml receiver tube • Proceed with step 2 "**DNA elimination**"

1b	**Manual sample grinding**
	• Grinding of the starting material by using a pestle and liquid nitrogen • Transfer the resulting powder in a 2.0 ml receiver tube *Note : do not thaw the sample* • Add 900 µl of DTT supplemented **Lysis Buffer LT** (shake gently before use) • Incubate under continuous shaking at room temperature until having a homogeneous lysate

Note: passing the lysate through a gauge 20 needle improves the RNA extraction yield. (Shearing occurs and DNA breaks down)

Steps 2 to 6 →

2	**DNA elimination** (and beads removal where necessary) • Centrifuge 2 min. at max. speed • Transfer carefully approx. 800 µl of the supernatant into a new 2.0 ml collection tube (not supplied) • Add 500 µl of >96 % ethanol into the new 2.0 ml collection tube • Mix entirely by pipetting

3	**RNA adsorption to the RNA Filter** • Transfer 750 µl of the solution from the previous step into the center of the **RNA Filter** from a **RNA Filter Set** (filter inserted in a tube) • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g • Discard the flow-through and put the **RNA Filter** back into the tube

4	**RNA washing, step I** • Add 600 µl of **Wash Solution M1** to the **RNA Filter** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and the Receiver tube • Insert the **RNA Filter** into a new **Receiver Tube GS**

5	**RNA washing, step II** • Add 700 µl of **Wash Solution M2** to the **RNA Filter** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and put the **RNA Filter** back into the **Receiver Tube GS** • Repeat 1 X the washing-centrifugation step • Discard the flow-through and put the **RNA Filter** back into the **Receiver Tube GS** • Centrifuge 4 min. at max. speed, to remove remaining ethanol

6	**Elution of total RNA** • Insert the **RNA Filter** into a RNase-free **Elution Tube** • Add 30 – 60 µl of **Elution Buffer KL** (depending on desired yield and concentration) • Incubate 2 min. at room temperature • Centrifuge 1 min. at 11000 x g • Discard the **RNA Filter** and place immediately the **Elution Tube** with eluted RNA on ice

Eluted RNA is ready for down-stream applications and can directly be used for Northern blots, dot blots, RT-PCR, DDRT-PCR, construction of cDNA libraries, etc., or can be stored at -80 °C for several weeks.

Note: RNA elution can also be achieved with RNase-free ddH_2O.

IX. Variant I: simultaneous extraction of total RNA and proteins

Proteins may be recovered from the flow-through of:

- Step 4 "**RNA adsorption to the RNA Filter**" of protocol 1. (page 5)
- Step 3 "**RNA adsorption to the RNA Filter**" of protocol 3. (page 11)

	Protein precipitation
1	• Add 3 volume of ice cold acetone to the flow-through • Vortex • Centrifuge 10 min. at 4 °C, at 11000 x g • Discard the supernatant (do not remove the pellet)

	Protein washing
2	• Add 500 µl of cold >96 % ethanol • Centrifuge 4 min. at 4 °C, at max. speed • Discard the supernatant (do not remove the pellet)

	Protein resuspension
3	• Resuspend the pellet/ the proteins in an appropriate buffer solution suitable for further applications (e.g.: Laemmli buffer then heat 5 min. at 99 °C.)

Caution: Never do a trichloroacetic acid (TCA) precipitation (risk of gas intoxication!)

X. Variant II: simultaneous extraction of total RNA and DNA in protocol 1

- DNA may be recovered from the **DNA Filter** of step 3 "**DNA elimination**" of protocol 1. (page 5)

1	**DNA washing, step I** • Insert the **DNA Filter** in a new 2.0 ml receiver tube (not supplied) • Add 600 µl of **Wash Solution M1** to the **DNA Filter** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and the Receiver tube • Insert the **DNA Filter** into a new receiver tube
2	**DNA washing, step II** • Add 700 µl of **Wash Solution M2** to the **DNA Filter** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and put the **DNA Filter** back into the receiver tube • Repeat 1 X the washing-centrifugation step • Discard the flow-through and put the **DNA Filter** back into the receiver tube • Centrifuge 4 min. at max. speed, to remove remaining ethanol
3	**Elution of genomic DNA** • Insert the **DNA Filter** into a 1.5 ml elution tube • Add 40 – 100 µl of **Elution Buffer KL** (depending on desired yield and concentration) • Incubate 2 min. at room temperature • Centrifuge 1 min. at 11000 x g • Discard the **DNA Filter** and place the elution tube with eluted DNA at 4 °C *Note: DNA elution can also be achieved with ddH_2O.*

Note: this protocol requires a larger amount of tubes and solutions. Using this protocol reduce the total number of RNA extractions.

XI. Variant III: RNA purification from aqueous phase of Trizol

1	**DNA elimination** • In a 2.0 ml reaction tube, add to up to 350 µl of Trizol aqueous phase an equal amount of DTT supplemented **Lysis Buffer LT** (shake gently before use) • Mix entirely by pipetting • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g • Transfer the supernatant into a new 2.0 ml receiver tube
2	**RNA adsorption to the RNA Filter** • Add 1 volume of >96 % ethanol to the supernatant from previous step • Mix entirely by pipetting • Transfer the mixture into the center of the **RNA Filter** from a **RNA Filter Set** (filter inserted in a tube) • Incubate 1 min. at room temperature • Centrifuge 1 min. at 11000 x g • Discard the flow-through and put the **RNA Filter** back into the receiver tube *Note: if the supernatant + ethanol volume exceeds 700 µl, operate in successive centrifugation steps by using the same RNA Filter.*
3	**RNA washing, step I** • Add 600 µl of **Wash Solution M1** to the **RNA Filter** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and the Receiver tube • Insert the **RNA Filter** into a new **Receiver Tube GS**
4	**RNA washing, step II** • Add 700 µl of **Wash Solution M2** to the **RNA Filter** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and put the **RNA Filter** back into the **Receiver Tube GS** • Repeat 1 X the washing-centrifugation step • Discard the flow-through and put the **RNA Filter** back into the **Receiver Tube GS** • Centrifuge 4 min. at max. speed, to remove remaining ethanol
5	**Elution of total RNA** • Insert the **RNA Filter** into a RNase-free **Elution Tube** • Add 40 – 100 µl of **Elution Buffer KL** (depending on desired yield and concentration) • Incubate 2 min. at room temperature • Centrifuge 1 min. at 11000 x g • Discard the **RNA Filter** and place immediately the **Elution Tube** with eluted RNA on ice

Eluted RNA is ready for down-stream applications and can directly be used for Northern blots, dot blots, RT-PCR, DDRT-PCR, construction of cDNA libraries, etc., or can be stored at -80 °C for several weeks.

Note: RNA elution can also be achieved with RNase-free ddH_2O.

XII. Variant IV: RNA isolation from liquids

Depending on sample characteristics (contaminated or not contaminated by DNA), start with step 1a or 1b

1a	**Sample preparation** (sample not contaminated by DNA)
	• Transfer the required amount(*) of DTT supplemented **Lysis Buffer LT** (shake gently before use) into an empty **DNA Filter** (filter inserted in a tube)
	(*) for samples with a volume of 100 µl: 350 µl of DTT supplemented **Lysis Buffer LT**. for samples with a volume of 200 µl: 700 µl of DTT supplemented **Lysis Buffer LT**.
	• Centrifuge 2 min. at 13400 x g • Discard the **DNA Filter** • Add the sample (100 µl or 200 µl, as the case may be) to the tube containing the flow-through • Proceed with step 2 "**RNA adsorption to the RNA Filter**"

1b	**Sample preparation** (sample contaminated by DNA)
	• Add the required amount(*) of DTT supplemented **Lysis Buffer LT** (shake gently before use) to the sample
	(*) for samples with a volume of 100 µl: 350 µl of DTT supplemented **Lysis Buffer LT**. for samples with a volume of 200 µl: 700 µl of DTT supplemented **Lysis Buffer LT**.
	• Mix thoroughly by pipetting • Transfer the entire mixture (including possible precipitates) into a **DNA Filter** (filter inserted in a tube) • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g *Note: if the volume of the mixture exceeds 700 µl, operate in successive centrifugation steps, by using the same DNA Filter.* • Discard the **DNA Filter**

Steps 2 to 5 →

2	**RNA adsorption to the RNA Filter**
	• Add the required amount(*) of ethanol >96 % to the receiver tube of the previous step (*) for samples with a volume of 100 µl: 250 µl of >96 % ethanol. for samples with a volume of 200 µl: 500 µl of >96 % ethanol. • Mix thoroughly by pipetting • Transfer the entire mixture into a **RNA Filter** (filter inserted in a tube) • Incubate 1 min. at room temperature • Centrifuge 2 min. at 11000 x g • Discard the flow-through and put the **RNA Filter** back into the receiver tube *Note: if the mixture volume exceeds 700 µl, operate in successive centrifugation steps by using the same RNA Filter.*

3	**RNA washing, step I**
	• Add 600 µl of **Wash Solution M1** to the **RNA Filter** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and the Receiver tube • Insert the **RNA Filter** into a new **Receiver Tube GS**

4	**RNA washing, step II**
	• Add 700 µl of **Wash Solution M2** to the **RNA Filter** • Centrifuge 1 min. at 11000 x g • Discard the flow-through and put the **RNA Filter** back into the **Receiver Tube GS** • Repeat 1 X the washing-centrifugation step • Discard the flow-through and put the **RNA Filter** back into the **Receiver Tube GS** • Centrifuge 4 min. at max. speed, to remove remaining ethanol

5	**Elution of total RNA**
	• Insert the **RNA Filter** into a RNase-free **Elution Tube** • Add 40 – 100 µl of **Elution Buffer KL** (depending on desired yield and concentration) • Incubate 2 min. at room temperature • Centrifuge 1 min. at 11000 x g • Discard the **RNA Filter** and place immediately the **Elution Tube** with eluted RNA on ice

Eluted RNA is ready for down-stream applications and can directly be used for Northern blots, dot blots, RT-PCR, DDRT-PCR, construction of cDNA libraries, etc., or can be stored at -80 °C for several weeks.

Note: RNA elution can also be achieved with RNase-free ddH_2O.