RNA Mini Kit

Data sheet

Order No. BS 67.311.0010 10 Preparations
Order No. BS 67.311.0050 50 Preparations
Order No. BS 67.311.0250 250 Preparations

(For research only)

Batch No.:

Best before:

Appearance: clear liquid

Colour: transparent

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RNA Mini Kit

Description

Bio&SELL RNA Mini kit is a method for the efficient isolation of total RNA from different starting materials (tissues, cells, bacteria, plants, etc.) as well as from different amounts of starting materials. The particular advantages of this product are its ease of use, the absence of phenol, and working outside of the hood.

Kit components (store at room temperature)

	10 preparations	50 preparations	250 preparations
Lysis Solution SM	6 ml	30 ml	125 ml
Washing Solution IT	3 ml (final volume 6 ml)	15 ml (final volume 30 ml)	70 ml (final volume 140 ml)
Washing Solution MT	2 ml (final volume 10 ml)	8 ml (final volume 40 ml)	40 ml (final volume 200 ml)
RNase-free water	1.5 ml	6 ml	2 x 15 ml
Spin filter E (blau)	10	50	5 x 50
Spin filter S (viol.)	10	50	5 x 50
Receiver tubes 2.0 ml	50	5 x 50	25 x 50
Elution tubes 1.5 ml	10	50	5 x 50

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Important steps before starting

Add 96-99.8% ethanol to Wash Buffer IT and MT. The corresponding volumes depends on the kit size, it can be found in the following table. Mix thoroughly and keep the bottle always tightly closed.

	10 Preparations	50 Preparations	250 Preparations
Washing Solution IT	**Add 3 ml** 96 – 99.8% ethanol	**Add 15 ml** 96 – 99.8% ethanol	**Add 70 ml** 96 – 99.8% ethanol
Washing Solution MT	**Add 8 ml** 96 – 99.8% ethanol	**Add 32 ml** 96 – 99.8% ethanol	**Add 160 ml** 96 – 99.8% ethanol

- Ensure that the Washing Solution IT and Washing Solution MT have been prepared according to the instruction.
- Centrifugation steps should be performed at room temperature.
- Avoid freezing and thawing of starting materials.

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Components not included in the kit

- Optional: DNase I
- Optional (bacteria protocol): Lysozyme
- Bacteria protocol: TE Buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0)
- ddH_2O
- Reaction tubes
- 70% ethanol
- 96 – 99.8% ethanol

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Safety instructions

Please handle all materials and reagents included in the kit carefully. Always wear gloves and avoid skin contact!
In case of contact with eyes or skin, rinse immediately with plenty of water!
Please read each section carefully to ensure your own safety and ease of implementation. Follow all safety instructions in this brochure. Please also note instructions and information.

For single use
Do not use a component a second time!

Attention!

- Drinking or eating of the kit components are strictly prohibited!
- If the buffer bottles are damaged or leaking, wear gloves and safety goggles when disposing the bottles to avoid injury.
- This kit can be used with potentially infectious samples. Therefore, all liquid wastes must be considered as potentially infectious and must be handled and discarded according to local safety regulations.
- Please observe the federal, state and local safety and environmental regulations.
- Follow the usual precautions for working with extracted nucleic acids.
- All materials and reagents used for DNA or RNA isolation should be free of DNases and RNases.

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Attention!
Do not add any bleach or acidic components to the waste after sample preparation.

►►► Notes:
In case of an emergency: Medical information in English and German can be obtained 24 hours a day.
Telephone numbers of the Poison Information Centers:
Munich +49 (0)89 19240
Vienna 01/406 43 43
Zurich + 41 44 251 51 51 (for Switzerland: 145)

For further information please request the safety data sheet.

GHS-Classification

Component	Hazardous content	GHS Symbol	H statements	P statements	EUH
Lysis buffer SM	Guanidinium thiocyanate 25-50%	 Hazard	302, 314, 412	101, 102, 103, 260, 303 + 361 + 353, 305 + 351 + 338, 310, 405, 501	032
Washing solution IT	Guanidinium thiocyanate 50-100%	 Hazard	302, 314, 412	101, 102, 103, 260, 303 + 361 + 353, 305 + 351 + 338, 310, 405, 501	032

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Hazard statements

302	Harmful if swallowed.
314	Causes severe skin burns and eye damage
412	Harmful to aquatic life with long lasting effects

Precautionary statements

101	If medical advice is needed, have product container or label at hand
102	Keep out of reach of children
103	Read label before use
260	Do not breathe dust/fume/gas/mist/vapours/spray
310	Immediately call a POISON CENTER (Munich: 089-19240, Vienna: 01-406 43 43) or an doctor
405	Store locked up
501	Dispose of contents/container in accordance with local and national legislation.
303 + 361 + 353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower
305 + 351 + 338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

EUH statements

032	Contact with acids liberates very toxic gas.

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General notes and safety recommendations on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases have to be reduced to a minimum in accordance with the following recommendations:

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contaminations from surface of the skin or from dusty laboratory equipment.
- Change gloves frequently and keep tubes closed.
- Keep isolated RNA on ice.
- Reduce preparation time as much as possible.
- Use only sterile, disposable polypropylene tubes throughout the procedure (these tubes are generally RNase-free.)
- Non-disposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. You can also take chloroform-resistant plasticware rinsed with chloroform to inactivate RNases.
- All glassware should be treated before use to ensure that it is RNase-free. Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240°C for four or more hours before use. Autoclaving alone will not inactivate many RNases completely. Oven baking inactivates RNases and ensures that no other nucleic acids (such as plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1 % DEPC (diethyl pyrocarbonate). The glassware has to be immersed in 0,1 % DEPC solution for 12 hours at 37°C and then it has to be autoclaved or heated to 100°C for 15 min to remove residual DEPC.
- Electrophoresis tanks should be cleaned with detergent solution (e.g. 0.5 % SDS), thoroughly rinsed with RNase-free water, rinsed with ethanol and finally allowed to dry.

- All buffers have to be prepared with DEPC-treated RNase-free ddH_2O.
- Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification will be performed.
- Do not use equipment, glassware and plasticware employed for other applications which might introduce RNase contaminations in the RNA isolation.

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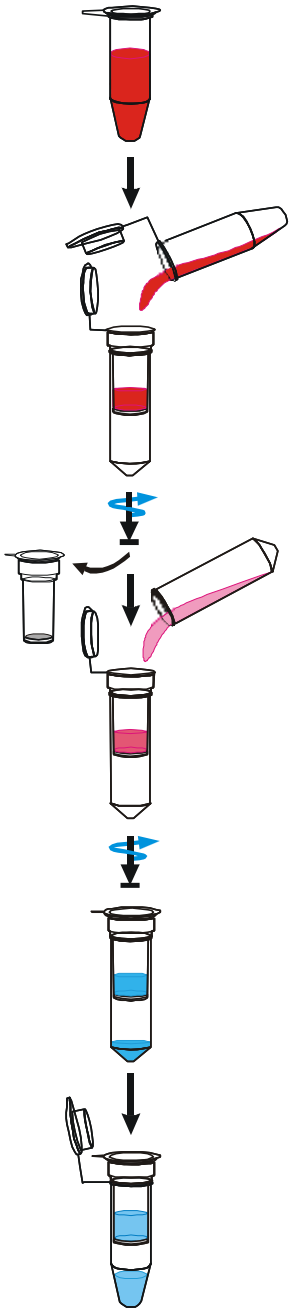
RNA Mini Kit

Operating mode



Homogenisation/Lysis of sample

Selective removal of genomic DNA

Selective binding of RNA

Washing of bound RNA

Elution of RNA

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Protocol 1: RNA extraction from tissue samples (up to 20 mg)

Important:

Please note that up to 20 mg of tissue samples can be processed.

Avoid freezing and thawing of tissue samples!

1. Homogenization of starting material

Note:
To maximize the final yield of total RNA a complete homogenization of tissue sample is important!

For the homogenization of tissue sample it is possible to use commercially available rotor-stator homogenizer or bead mills. It is also possible to disrupt the starting material using mortar and pestle in liquid nitrogen and grind the tissue sample to a fine powder.

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A. Homogenization of the tissue sample using a rotor-stator homogenizer

1. Transfer the weighed amount of fresh or frozen starting material in a suitable reaction vessel for the homogenizer.
2. Add 450 µl Lysis Solution SM.
3. Homogenize the sample.
4. Transfer the homogenized tissue sample into a 1.5 ml reaction tube and place the sample under Lysis Solution SM for longer storage at –20°C or use the sample immediately for isolation of total RNA following the protocol step 2.

B. Disruption of the tissue sample using a mortar and pestle and liquid nitrogen

1. Transfer the weighed amount of fresh or frozen starting material under liquid nitrogen and grind the material to a fine tissue powder.
2. Transfer the powder into a 1.5 ml reaction tube.
   Don’t allow the sample to thaw!
3. Add 450 µl Lysis Solution SM and incubate the sample for appropriate time for a further lysis under continuous shaking.
4. Finally place the sample under Lysis Solution SM for longer storage at – 20°C or use the sample immediately for isolation of total RNA following protocol step 2.

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2. Clarifying of the sample

After lysis spin down unlysed material by centrifugation at maximum speed for 1 minute. Place a Spinfilter E into a 2.0 ml Receiver Tube. Transfer the supernatant of the lysed sample onto the Spinfilter E. Centrifuge at 10,000 x g (12,000 rpm) for 2 minutes. Discard the Spinfilter E.
Do not discard the filtrate, because the filtrate contains the RNA!

Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolonge the centrifugation time.

3. Binding of the sample

1. Place a Spinfilter S into a new 2.0 ml Receiver Tube.
2. Add an equal volume (appr. 400 µl) of 70 % ethanol to the filtrate from step 2.
3. Mix the sample by pipetting sometimes up and down.
4. Transfer the sample onto the Spinfilter S.
5. Centrifuge at 10,000 x g (12,000 rpm) for 2 minutes.

Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolonge the centrifugation time.

Discard the 2.0 ml Receiver Tube with filtrate and place the Spinfilter S into a new 2.0 ml Receiver Tube.

4. Washing of the sample I

Open the Spinfilter S and add 500 µl Washing Solution IT, close the cap and centrifuge at 10,000 x g (12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spinfilter S into a new 2.0 ml Receiver Tube.

5. Washing of the sample II

Open the Spinfilter S and add 700 µl Washing Solution MT, close the cap and centrifuge at 10,000 x g (12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spinfilter S into a new 2.0 ml Receiver Tube.

6. Drying of the column

Centrifuge at 10,000 x g (12,000 rpm) for 2 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

7. Elution of the RNA

1. Place the Spinfilter S into a 1.5 ml Elution Tube.
2. Carefully open the cap of the Spinfilter S and add 30 - 80 µl RNase-free water.
3. Incubate at room temperature for 1 minute. Centrifuge at 6,000 x g (8,000 rpm) for 1 minute.

Note: Depending on the extracted yield or the needed concentration of total RNA you can also elute with different volumes of RNase-free water.
A lower volume of RNase-free water increases the concentration of RNA and a higher volume of RNase-free water leads to an increased yield but a lower concentration of total RNA. Please note, that the minimum of RNase- free water should be 20 µl.

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Protocol 2: RNA extraction from eucaryotic cells (5 x 10^6 cells)

Important: Please note that up to 5 x 10^6 cells can be processed.

1. Lysis of the cells

Add 400 µl Lysis Solution SM to the cell pellet. Incubate for 2 minutes at room temperature. Resuspend the cell pellet completely by pipetting up and down. Incubate the sample for further 3 minutes at room temperature.

Note: To maximize the final yield of total RNA a complete disruption and lysis of the cell pellet is important! No cell clumps should be visible after lysis step.

2. Clarifying of the sample

Place a Spinfilter E into a 2.0 ml Receiver Tube. Transfer the lysed sample onto the Spinfilter E. Centrifuge at 10,000 x g (12,000 rpm) for 2 minutes. Discard the Spinfilter E. **Do not discard the filtrate, because the filtrate contains the RNA!**

Note*:* If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolonge the centrifugation time.

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3. Binding of the sample

Place a Spinfilter S into a new 2.0 ml Receiver Tube. Add an equal volume (appr. 400 µl) of 70 % ethanol to the filtrate from step 2. Mix the sample by pipetting sometimes up and down. Transfer sample onto the Spinfilter S. Centrifuge at 10,000 x g (12,000 rpm) for 2 minutes.

Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolonge the centrifugation time. Discard the 2.0 ml Receiver Tube with filtrate and place the Spinfilter S into a new 2.0 ml Receiver Tube.

4. Washing of the sample I

Open the Spinfilter S and add 500 µl Washing Solution IT, close the cap and centrifuge at 10,000 x g (12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spinfilter S into a new 2.0 ml Receiver Tube.

5. Washing of the sample II

Open the Spinfilter S and add 700 µl Washing Solution MT, close the cap and centrifuge at 10,000 x g (12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spinfilter S into a new 2.0 ml Receiver Tube.

6. Drying of the column

Centrifuge at 10,000 x g (12,000 rpm) for 3 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

7. Elution of the RNA

Place the Spinfilter S into a 1.5 ml Elution Tube. Carefully open the cap of the Spinfilter S and add 30 - 80 µl RNase-free water. Incubate at room temperature for 1 minute. Centrifuge at 6,000 x g (8,000 rpm) for 1 minute.

Note:
Depending on the extracted yield or the needed concentration of total RNA you can also elute with different volumes of RNase-free water. A lower volume of RNase-free water increases the concentration of RNA and a higher volume of RNase-free water leads to an increased yield but a lower concentration of total RNA. Please note, that the minimum of RNase-free water should be 20 µl.

Protocol 3: RNA extraction from bacterial cells

Important:
We recommend a preincubation of bacterial cells with Lysozyme or optionally other bacterial lysis proteins.
Stock solution of Lysozyme for gram(-) bacteria: 20 mg/ml in water; storage of Lysozyme stock solution in aliquots at –20°C.
Stock solution of Lysozyme for gram(+) bacteria: 50 mg/ml in water; storage of Lysozyme stock solution in aliquots at –20°C.

Prepare TE-Buffer (10mM Tris HCl / 1 mM EDTA; pH 8.0)

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1. Preliminary step

Spin down the bacterial cells (max. 1 x 10^9 cells) by centrifugation at 5,000 x g for 2 –5 minutes. Discard the supernatant as complete as possible.

A) For **gram(-) bacteria** resuspend the cell pellet in 100 µl TE-Buffer and add 2 µl of the corresponding Lysozyme stock solution. Pipette sometime up and down; the solution should become clear or viscous.
B) For **gram(+) bacteria** resuspend the cell pellet in 100 µl TE-Buffer and add 6 µl of the corresponding Lysozyme stock solution. Pipette sometimes up and down; incubate until the solution becomes clear or viscous.

Note: The amount of Lysozyme and also the essential time for incubation may need to be diversified depending on bacterial strains. Read also the guideline of the Lysozyme supplier. A complete destruction of bacterial cell walls is important.

2. Lysis of the cells

Add 450 µl Lysis Solution SM to the sample and vortex vigorously or pipette sometimes up and down. Incubate the sample for further 3 minutes at room temperature.

Note: To maximize the final yield of total RNA a complete disruption and lysis of the cell pellet is important! No cell clumps should be visible after lysis step.

3. Clarifying of the sample

Place a Spinfilter E into a 2.0 ml Receiver Tube. Transfer the lysed sample onto the Spinfilter E. Centrifuge at 10,000 x g (12,000 rpm) for 2 minutes. Discard the Spinfilter E. **Do not discard the filtrate, because the filtrate contains the RNA!**

Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolonge the centrifugation time.

4. Binding of the sample

Place a Spinfilter S into a new 2.0 ml Receiver Tube. Add an equal volume (appr. 600 µl) of 70 % ethanol to the filtrate from step 2. Mix the sample by pipetting sometimes up and down. Transfer 650 µl of the sample onto the Spinfilter S. Centrifuge at 10,000 x g (12,000 rpm) for 1 minute. Discard the 2.0 ml Receiver Tube and place the Spinfilter S into a new 2.0 ml Receiver Tube. Load the residual sample on the Spinfilter S and centrifuge again at 10,000 x g (12,000 rpm) for 1 minute.

Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolonge the centrifugation time.

Discard the 2.0 ml Receiver Tube with filtrate and place the Spinfilter S into a new 2.0 ml Receiver Tube.

5. Washing of the sample I

Open the Spinfilter S and add 500 µl Washing Solution IT, close the cap and centrifuge at 10,000 x g (12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spinfilter S into a new 2.0 ml Receiver Tube.

6. Washing of the sample II

Open the Spinfilter S and add 700 µl Washing Solution MT, close the cap and centrifuge at 10,000 x g (12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spinfilter S into a new 2.0 ml Receiver Tube.

7. Drying of the column

Centrifuge at 10,000 x g (12,000 rpm) for 3 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

8. Elution of the RNA

Place the Spinfilter S into a 1.5 ml Elution Tube. Carefully open the cap of the Spinfilter S and add 30 - 80 µl RNase-free water. Incubate at room temperature for 1 minute. Centrifuge at 6,000 x g (8,000 rpm) for 1 minute.

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Troubleshooting

Problem/ probable cause	Comments and suggestions
Clogged Spin Filter • insufficient disruption or homogenisation	After lysis centrifuge lysate to pellet debris and continue with the protocol using the supernatant. Reduce amount of starting material.
Little or no total RNA eluted • insufficient disruption or homogenisation • incomplete elution	Reduce amount of starting material. Overloading reduces yield! Prolong the incubation time with RNase-free water to 5 minutes or repeat elution step once again.
DNA contamination • too much starting material • incorrect lysis of starting material	Reduce amount of starting material. Use the recommended techniques for lysis of cell pellet. **Perform DNase digest of the eluate containing the total RNA or perform a on column DNase digest step after binding of the RNA on Spinfilter S!**
Total RNA degraded • RNA source inappropriately handled or stored • RNase contaminations of solutions, Receiver Tubes etc.	Ensure that the starting material is fresh! Ensure that the protocol, especially the first steps, have been performed quickly. Use sterile, RNase-free filter tips. Before every preparation clean up the pipet, the devices and the working place. Always wear gloves!
Total RNA does not perform well in downstream-applications (e.g. RT-PCR) • ethanol carryover during elution • salt carryover during elution	Increase time for removing of ethanol. Ensure that Washing Solution IT and Washing Solution MT are at room temperature. Check up Washing Solutions for salt precipitates. If there are any precipitates dissolve these precipitates by careful warming.

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Calculation of the centrifugal force (relative centrifugal force, rcf) in [g], based on rpm (revolutions per minute, rpm)

RCF (g) = $1{,}19 \cdot 10^{-5}$ x $(\text{rpm})^2$ x R

R = radius of the rotor in cm (Attention: radius, not diameter!)

Example: calculation of centrifugal force at 10,000 rpm in a centrifuge with rotor radius 9 cm

RCF = $1{,}19 \cdot 10^{-5}$ x $(10.000)^2$ x 9

RCF = 10.710 x g

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Technical Data

All Bio&SELL products undergo comprehensive quality controls. This ensures that they function properly when used as prescribed. The components of each Bio&SELL RNA Mini Kit were tested in the isolation of total RNA from tissue samples and the extracted RNA was analyzed on the Agilent Bioanalyzer.

Shipment: at room temperature

Storage: **The Bio&SELL RNA Mini Kit should be stored dry, at room temperature (14 – 25°C)** and is stable for at least 6 months under these conditions. Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by careful warming.

Safety precautions

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.

Product use and warranty

The user is responsible to validate the performance of Bio&SELL kits for any particular use, since the performance characteristics of our kits have not been validated for any specific application. Bio&SELL kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA' 88 regulations in the U.S. or equivalents in other countries. All products sold by Bio&SELL GmbH are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

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RNA Mini Kit

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- **Fast protocols**

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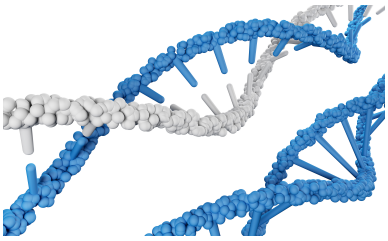

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