Extracta Plus RNA

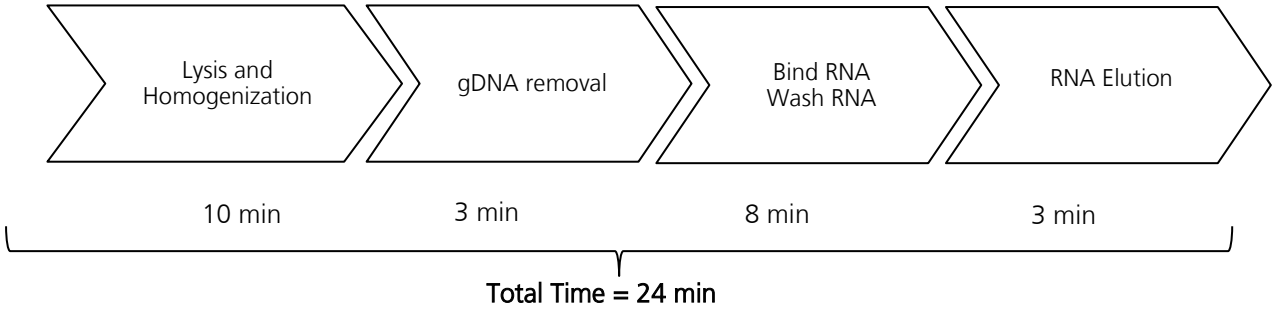
Cat. No. 95214-010 Size: 10 reactions **Store at 15°C to 25°C**
95214-050 50 reactions

Description

The Extracta Plus RNA kits offer rapid purification of Total RNA from animal cells or tissues. The Extracta Plus Spin Column workflow enables simultaneous processing of multiple samples in just 25 mins to yield purified total RNA suitable for a range of downstream applications such as next-generation sequencing, RT-(q)PCR, cDNA synthesis and microarrays. The kit includes specially designed Extracta Plus DNA removal columns for effective removal of genomic DNA contamination, providing a convenient solution for applications that are sensitive to low amounts of DNA contamination such as RT-qPCR.

The Extracta Plus RNA protocol isolates all RNA molecules >200 nucleotides in length, enriching for mRNA since most RNAs <200 nucleotides are selectively excluded (such as 5.8S rRNA, 5S rRNA and tRNAs). High quality RNA is eluted in nuclease-free water for immediate use in downstream applications, or storage at -20°C or -80°C for later use.

Workflow Overview



Components

	Volume	
Component Description	**10 Reactions**	**50 Reactions**
Buffer EPRL	6 ml	45 ml
Buffer EPRW1	7 ml	45 ml
Buffer EPRW2	3 ml	11 ml
RNase-free water	10 ml	10 ml
Extracta Plus Spin Column	10 piece	50 piece
Extracta Plus DNA Removal Column	10 piece	50 piece
Collection Tubes 1.5 ml	10 piece	50 piece
Collection Tubes 2 ml	10 piece	50 piece

Storage and Stability

Store Extracta Plus Spin Columns and buffers at room temperature (15-25°C).

Additional reagents and materials that are not supplied

- 14.3 M β-mercaptoethanol (β-ME) or freshly prepared 2 M dithiothreitol (DTT) stock solution
- Ethanol (96–100%)
- 70% EtOH in water

General Guidelines

- Suitable personal protective equipment should be worn when handling chemicals. Be sure to read all relevant safety information and reagent safety data sheets before beginning.
- Always use PCR tubes, microcentrifuge tubes, and pipette tips that are certified sterile, DNase- and RNase-free.
- All steps should be performed at room temperature. During the procedure, work quickly to avoid RNA degradation.
- All centrifugation steps should be performed at 20-25°C in a standard microcentrifuge. Ensure that the microcentrifuge does not cool below 20°C.
- Read the entire protocol before beginning. Take note of the recommended input sample amounts and different extraction procedures for cultured cells and tissues.

Point in protocol where procedure can be stopped and stored at appropriate conditions outlined

Take note of recommendations in protocol

Use caution to obtain the best results when performing protocol

Before You Begin

- Wipe down work areas and pipettes with an RNase and DNA cleaning product.
- This protocol is suitable for fresh or frozen cell pellets (stored at -70°C). Allow frozen cell pellets to thaw slightly before lysis.
- Prepare Buffer EPRW2: Before first use, add 4 volumes of ethanol (96-100%) indicated on the bottle to the buffer concentrate and shake to mix thoroughly.
- Buffer EPRL may form a precipitate during storage. If necessary, warm solution until the precipitate has fully dissolved then place at room temperature before use.
- For RNA extraction from cell lines with high RNase content or from tissues, β-ME or DTT should be added to buffer EPRL before use as specified in the protocols below.

DO NOT add bleach or acidic solutions directly to the sample preparation waste. Buffers EPRL and EPRW1 contain guanidine salts, which can form highly reactive compounds when combined with bleach (sodium hypochlorite). Any spills should be cleaned with a suitable laboratory detergent and water. If the solution contains infectious agents, clean the area with detergent and water before disinfecting with 1% (v/v) bleach.

If using β-mercaptoethanol (β-ME), dispense in a fume hood and wear appropriate personal protective equipment.

Protocol

This protocol is designed for the purification of total RNA from animal cells and tissues. It is important not to overload the Extracta Plus DNA Removal Column or Extracta Plus Spin Column as this can result in carryover of gDNA and reduction in purity and yield of RNA. Starting material should be quantified before input into the Extracta Plus RNA protocol by either counting cells or weighing tissue. The maximum recommended input amount of cells or tissue is given for each protocol below, however the RNA content will vary between cell types. Detailed guidance on starting input sample amounts is given in **Appendix A (page 8)**.

The Extracta Plus RNA procedure isolates all RNA molecules >200 nucleotides and therefore is selective for mRNA. Highly abundant RNAs <200 nucleotides such as 5.8S rRNA, 5S rRNA and tRNAs (that together comprise 12-20% total RNA) are selectively excluded during the procedure. An alternative protocol for the purification of total RNA containing small RNA from cultured cells is provided in **Appendix B (page 9)** The Extracta Plus RNA kit is not suitable for the purification of microRNA (miRNA) from animal or human tissues.

Lysis, homogenization and gDNA removal

Purification of Total RNA from Animal Cells

This protocol is suitable for a maximum of 1 x 10^7 cells. RNA content can vary by cell type, see **Appendix A (page 8)** for detailed guidance on maximum input amounts for different cell types.

Optional: When purifying RNA from cell lines with high RNase content, add β -ME or DTT solution to Buffer EPRL before use. Either add 10 µl β -ME or 20 µl of 2 M DTT solution per 1 ml Buffer EPRL. Pre-mixed solutions can be stored at room temperature for up to 1 month.

1. **For cells grown in suspension**: Pellet the appropriate number of cells by centrifuging for 5 min at 300 x *g*. Carefully remove all supernatant and proceed to step 2.

   For cells grown in monolayer: Aspirate the cell culture medium and wash the cells in PBS. Add trypsin (0.1-0.25% in PBS) and incubate at 37°C until the cells detach. Inactivate trypsin with the addition of cell culture medium (containing serum), transfer to a centrifuge tube and pellet cells by centrifuging for 5 min at 300 x *g*. Remove all supernatant and proceed to step 2.

   For direct lysis of cells grown in monolayer: Aspirate the cell culture medium and add Buffer EPRL (350 µl for dishes <6 cm diameter or 600 µl for dishes 6-10 cm diameter). Collect the lysate with a rubber policeman or cell scraper and pipette the lysate into a 1.5 ml microcentrifuge tube. Vortex or pipette to mix thoroughly until no cell clumps are visible and proceed directly to step 3.

2. Add buffer EPRL to the pelleted cells (350 µl for <5 x 10^6 cell or 600 µl for 5 x 10^6 – 1 x 10^7 cells). Vortex or pipette to mix thoroughly.
3. Pass the lysate through a 20-gauge needle (0.9 mm diameter) fitted on a RNase-free syringe at least 5 times to homogenize.

   It is important to ensure the lysate is completely homogenized. Incomplete homogenization can lead to reduced RNA yields and clogging of the Extracta Plus Spin Column. Other methods of homogenization such as using a rotor-stator homogenizer, bead milling or specialized spin-columns for homogenization are acceptable.

Homogenized cell lysates can be stored at -70°C for several months. Frozen lysates should be incubated at 37°C in a water bath until completely thawed and salts are dissolved before continuing with step 4. Prolonged incubation can compromise RNA integrity and should be avoided.

4. Transfer the homogenized lysate into an Extracta Plus DNA Removal Column (in a 2 ml collection tube) and centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm). Repeat the centrifugation until all of the liquid has passed through the membrane. **Keep the flow-through,** discard the spin column.

DO NOT discard the flow-through this contains the RNA that will subsequently be purified.

Proceed to Bind and Wash RNA steps

Purification of Total RNA from Animal Tissues

This protocol is suitable for a maximum of 30 mg fresh or frozen tissue or 15-20 mg tissue in a tissue or cell storage reagent such as RNAprotect (QIAGEN) or RNAlater (Thermo Scientific). Exceeding the maximum recommended input amount can lead to reduced RNA yield and purity and incomplete gDNA removal. More efficient DNA removal may be achieved with smaller input amounts.

For tissues with very high DNA content (e.g. spleen or thymus), treatment of the homogenized tissue with an RNase-free DNase reagent may be necessary. For very fibrous tissues (e.g. skeletal muscle, heart and skin), RNA yields may be reduced because of abundant contractile proteins, connective tissue and collagen.

1. Prepare a pre-mix of either β-ME or DTT with Buffer EPRL. Either add 10 µl β-ME or 20 µl of 2 M DTT solution per 1 ml Buffer EPRL (pre-mixed solutions can be stored at room temperature for up to 1 month).
2. Place the tissue in liquid nitrogen and grind thoroughly with a pestle and mortar. Transfer the tissue powder and liquid nitrogen into a pre-cooled 2 ml microcentrifuge tube. Allow the liquid nitrogen to evaporate but do not allow the tissue to thaw.

   Other methods of disruption and homogenization such as using a rotor-stator homogenizer or bead milling are also acceptable. For such protocols, do not freeze and grind the tissue. Instead, place the tissue in a suitable vessel for homogenization, add Buffer EPRL (as specified in step 3) directly to the tissue, disrupt and homogenize the tissue, then continue with step 5 of this protocol.

3. Immediately add Buffer EPRL plus β-ME or DTT: For <20 mg tissue add 350 µl Buffer EPRL, for 20- 30 mg tissue add 600 µl Buffer EPRL.

Tissues stabilized in a cell or tissue storage reagent may be more firm than fresh or frozen tissues. To aid lysis, 600 µl EPRL may be required for disruption and homogenization of ≤20 mg such stabilized tissue.

4. Pass the lysate through a 20-gauge needle (0.9 mm diameter) fitted on a RNase-free syringe at least 5 times to homogenize.

   Note: Specialized spin-columns for homogenization can be used as an alternative to homogenization using a needle and syringe. For such methods, transfer the lysate into the homogenization column (not provided) and centrifuge according to the manufacturer's instructions.

Homogenized tissue lysates can be stored at -70°C for several months. Frozen lysates should be incubated at 37°C in a water bath until completely thawed and salts are dissolved before continuing with step 5. Prolonged incubation can compromise RNA integrity and should be avoided.

5. Centrifuge the homogenized lysate for 3 min at maximum speed.

6. Transfer the supernatant into an Extracta Plus DNA Removal Column (in a 2 ml collection tube) and centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm). Repeat the centrifugation until all of the liquid has passed through the membrane. **Keep the flow-through,** discard the spin column.

DO NOT discard the flow-through- this contains the RNA that will subsequently be purified.

Proceed to Bind and Wash RNA steps

Bind and Wash RNA:

1. Add 1 volume of 70% EtOH to the flow-through and pipette to mix thoroughly.
   This volume is usually 300 µl or 600 µl. If some lysate volume was lost during homogenization, the volume of EtOH can be adjusted.
2. Assemble Extracta Plus RNA Spin Column by placing it in a 2 ml collection tube (provided). The collection tube will be re-used for step 3-6.
3. Transfer the sample/EtOH mixture, including any precipitate that may have formed, into the spin column and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow through.
   Throughout steps 3-6, take care to ensure the column does not come in contact with the flow-through.
4. Add 700 µl Buffer EPRW1 to the spin column and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow through.
5. Add 500 µl Buffer EPRW2 to the spin column and centrifuge for 15 s at ≥8000 x *g* (≥10,000 rpm). Discard the flow through.
6. Add 500 µl Buffer EPRW2 to the spin column and centrifuge for 2 min at ≥8000 x *g* (≥10,000 rpm). Discard the flow through.
7. Place the Extracta Plus Spin Column in a new 2 ml collection tube (provided) and centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm).

Elute RNA:

1. Place the Extracta Plus Spin Column in a new 1.5 ml collection tube (provided) and add 30- 50 µl RNase-free water to the spin column. Centrifuge for 1 min at ≥8000 x *g* (≥10,000 rpm) to elute RNA.

   <u>Optional:</u> If expected yield of RNA is > 30 µg, a second elution step can be carried out, either using another 30- 50 µl RNase-free water or re-using the eluate from the first elution.
   Re-using the eluate from the first elution will give a higher final RNA concentration but total RNA yield will be 15-30% less than if using a second volume of RNase-free water.

Purified RNA can be stored in RNase-free water at -70°C to -15°C for up to 1 year.

RNA Validation and Quantification

RNA concentration can be determined using a spectrophotometer by measuring the absorbance at 260 nm (A_{260}). The ratio of absorbance values at 260 nm and 280 nm (A_{260}/A_{280}) gives an estimate of RNA purity. Pure RNA has an A_{260}/A_{280} ratio in the range of 1.9-2.1 in 10 mM Tris-HCl, pH 7.5.

For very small amounts of RNA, more accurate quantification can be achieved with fluorometric or RT-qPCR methods.

Integrity and size distribution of total RNA can be determined by denaturing gel electrophoresis and ethidium bromide staining, or using a digital electrophoresis system such as Agilent 2100 Bioanalyzer or Agilent 2200 TapeStation. 28S rRNA and 18S rRNA should appear as sharp bands or peaks, at a ratio of approximately 2:1. Ribosomal RNA peaks that are not sharp or that are smeared towards smaller RNA sizes, indicate degradation of the RNA either before or after the Extracta RNA Plus procedure. The Agilent digital electrophoresis instruments provide an RNA integrity number (RIN) which is a measure of RNA integrity. Ideally, this should be close to 10 but in many cases it is influenced by how well the original sample was preserved.

Quality Control

Membrane control

The RNA-binding capacity was tested by determining the recovery of RNA from the membrane of the Spin Column.

As tested by PCR, no PCR inhibitors were released from the membrane.

Buffers

Conductivity and pH of buffers and solutions were tested and were within specifications.

Functional absence of RNase was checked for RNase-free water and Buffer EPRW2.

Extracta Plus DNA Removal Column

Extracta Plus DNA Removal Columns were functionally tested for DNA binding capacity.

Extracta Plus Spin Column

Extracta Plus Spin Columns were tested by preparation of total RNA from human cells and from 3 mg of tissue.

Integrity and size distribution of total RNA was checked by denaturing agarose gel electrophoresis and ethidium bromide staining.

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Appendix A: Guidance on starting input amounts

This section gives guidance on the expected RNA yield from various cell and tissue types. It is important not to overload the Extracta Plus DNA Removal Column or Extracta Plus Spin Column as this can result in carry over of gDNA and reduction in purity and yield of RNA. Starting material should be quantified before input into the Extracta Plus RNA protocol by either counting cells or weighing tissue. RNA content will also vary due to other factors such as species, development stage and growth conditions. The Extracta Plus RNA procedure isolates all RNA molecules >200 nucleotides and therefore is selective for mRNA. Highly abundant RNAs <200 nucleotides such as 5.8S rRNA, 5S rRNA and tRNAs (that together comprise 12-20% total RNA) are selectively excluded during the procedure.

The RNA content varies between cell lines, table 1 gives a general guide of the expected total RNA yields from common cell lines. The maximum recommended input for cultured cells is 1 x 10^7 cells. For cell lines not listed in table 1 or for which the user has no information about RNA content, it is recommended that no more than 3-4 x 10^6 cells are processed at first. This starting input amount could then be increased based on RNA yield and purity.

Cultured cell line (1 x 10^6 cells)	Average yield of total RNA (µg)
NIH/3T3	10
HeLa	15
COS7	35
LMH	12
Huh	15

Table 1: Expected yields of total RNA (> 200 nucleotides) from 1 x 10^6 cells using the Extracta Plus RNA kit.

Different tissue types will also vary in RNA content. The maximum recommended input of tissue is 30 mg for fresh or frozen tissue, or 15-20 mg of tissue in a tissue storage reagent. In general, a 3 mm cube of most animal tissue weighs approximately 30-35 mg. Table 2 gives guidance on the average yield of Total RNA from mouse/ rat tissues as an example. For tissues where the RNA content is unknown, it is recommended to start with 10 mg and then increase up to 30 mg for subsequent extractions depending on RNA yield and purity.

Mouse/ rat tissues (10 mg)	Average yield of total RNA (µg)
Embryo (13 day)	25
Brain	5-10
Heart	4-8
Kidney	20-30
Liver	40-60
Lung	10-20

Table 2: Expected yields of total RNA (>200 nucleotides) from 10 mg mouse/rat tissues using the Extracta Plus RNA kit.

Appendix B: Purification of Total RNA Containing Small RNAs from Cultured Cells

The standard Extracta RNA Plus protocol selects for RNA molecules >200 nucleotides in length. This section describes an alternative protocol for the purification of total RNA containing small RNAs, such as miRNA, from cultured animal and human cells. This protocol is not suitable for miRNA purification from animal or human tissues.

This protocol is suitable for a maximum of 5 x 10^6 cells.

Lysis, homogenization and gDNA removal

Optional: When purifying RNA from cell lines with high RNase content, add β-ME or DTT solution to Buffer EPRL before use. Either add 10 µl β-ME or 20 µl of 2 M DTT solution per 1 ml Buffer EPRL. Pre-mixed solutions can be stored at room temperature for up to 1 month.

1. **For cells grown in suspension**: Pellet the appropriate number of cells by centrifuging for 5 min at 300 x *g*. Carefully remove all supernatant and proceed to step 2.

   For cells grown in monolayer: Aspirate the cell culture medium and wash the cells in PBS. Add trypsin (0.1-0.25% in PBS) and incubate at 37°C until the cells detach. Inactivate trypsin with the addition of cell culture medium (containing serum), transfer to a centrifuge tube and pellet cells by centrifuging at for 5 min at 300 x *g*. Remove all supernatant and proceed to step 2.

2. Add buffer 350 µl EPRL to the pelleted cells. Vortex or pipette to mix thoroughly.

3. Pass the lysate through a 20-gauge needle (0.9 mm diameter) fitted on a RNase-free syringe at least 5 times to homogenize.

   It is important to ensure the lysate is completely homogenized. Incomplete homogenization can lead to reduced RNA yields and clogging of the Extracta Plus Spin Column. Other methods of homogenization such as using a rotor-stator homogenizer, bead milling or specialized spin-columns for homogenization are acceptable.

4. Transfer the homogenized lysate into an Extracta Plus DNA Removal Column (in a 2 ml collection tube) and centrifuge for 30 s at ≥8000 x *g* (≥10,000 rpm). Repeat the centrifugation until all of the liquid has passed through the membrane. **Keep the flow-through,** discard the spin column.

DO NOT discard the flow-through- this contains the RNA that will subsequently be purified.

Add 1.5 volumes of 100% Ethanol (usually 525 µl) to the flow-though and vortex to mix thoroughly.

Proceed immediately to step 2 of Bind and Wash RNA (page 5)