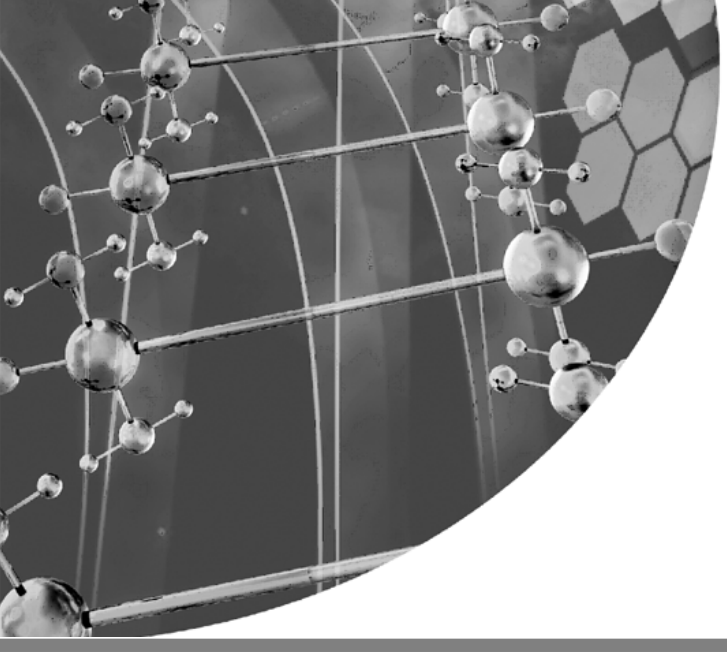

Isol-RNA Lysis Reagent Manual

Efficient lysis of a variety of tissues types before RNA isolation

Isol-RNA Lysis Reagent Manual, June 2007

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Product spefications

Product description

Isol-RNA Lysis Reagent is designed to facilitate lysis of fatty tissues and inhibit RNases and is used to prepare homogenates for RNA purification.

Product limitations

Isol-RNA Lysis Reagent is developed, designed, and sold for research purposes only. It is not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of the materials described in this text.

Tungsten carbide beads react with Isol-RNA Lysis Reagent and must not be used to disrupt and homogenize tissues.

Materials supplied

Isol-RNA Lysis Reagent	Order/ref. no.	Size
Isol-RNA Lysis Reagent	2302700	200 ml

Additional materials

To perform the protocols described in this manual, the following additional materials must be provided by the user:

For RNA isolation procedure

- Chloroform
- Isopropanol
- 75% ethanol
- RNase-free water
- Disposable gloves
- Refrigerated laboratory centrifuge or microcentrifuge (capable of 12,000 x *g*)
- For stabilization of RNA in tissues: liquid nitrogen or RNAlater™ RNA Stabilization Reagent (QIAGEN)
- Equipment for tissue disruption and homogenization. Thee following are recommended:
  - Rotor-stator homogenizer, such as the TissueRuptor (QIAGEN)
  - Bead-mill, such as the TissueLyser (QIAGEN)
- Kit for the RNA cleanup after the Isol-RNA procedure, such as RNeasy™ Kits (QIAGEN). Alternatively, RNA can be cleaned up with isopropanol precipitation (page 15)

For genomic DNA isolation procedure

- 75% ethanol
- 100% ethanol
- 0.1 M sodium citrate solution (0.1 M sodium citrate in 10% ethanol [p.a.])
- 8 mM NaOH
- 0.1 M HEPES (free base)
- 100 mM EDTA

For protein isolation procedure

- 100% ethanol
- Isopropanol
- Guanidine-ethanol solution (0.3 M guanidine-hydrochloride in 95% ethanol)
- Urea/DTT solution (10 M urea, 50 mM DTT in water). Dissolve urea in a small volume of water by stirring; weigh DTT just before use, dissolve in a small volume of water, and add to the urea solution while stirring; add water to adjust the final concentrations.

Shipping and storage

Isol-RNA Lysis Reagent is shipped at ambient temperature.

Isol-RNA Lysis Reagent should be stored dry at room temperature (15–25°C) or at 2–8°C.

Safety information

All due care and attention should be exercised in the handling of this product. We recommend all users of 5 PRIME products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines. Specifically, always wear a suitable lab coat, disposable gloves, and protective goggles when working with chemicals.

Additional safety information is available from www.5 PRIME.com in material safety data sheets (MSDSs) for 5 PRIME products and 5 PRIME product components.

Quality assurance

5 PRIME products are manufactured using quality chemicals and materials that meet our high standards. All product components are subjected to rigorous quality assurance testing process:

- **Component testing:** each component is tested to ensure the composition and quality meet stated specifications.
- **Performance testing:** each product is tested to ensure it meets the stated performance specification.

Additional quality information is available from www.5 PRIME.com including certificate of analysis sheets for 5 PRIME products and 5 PRIME product components.

Product warranty

5 PRIME is committed to providing products that improve the speed, ease-of-use and quality of enabling technologies.

5 PRIME guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use.

This warranty is in place of any other warranty or guarantee, expressed or implied, instituted by law or otherwise. 5 PRIME provides no other warranties of any kind, expressed or implied, including warranties of merchantability and fitness for a particular purpose. Under no circumstance shall 5 PRIME be responsible for any direct, indirect, consequential or incidental damages or loss arising from the use, misuse, results of use or inability to use its products, even if the possibility of such loss, damage or expense was known by 5 PRIME.

Protocols

Isol-RNA Lysis principle

Designed to facilitate lysis of fatty tissues and inhibit RNases, Isol-RNA Lysis Reagent is a monophasic solution of phenol and guanidine thiocyanate. After disrupting and homogenizing tissue samples in Isol-RNA Lysis Reagent, chloroform is added and the homogenate is separated by centrifugation. The organic (lower) contains and proteins, and the interphase contains DNA. The aqueous (upper) phase contains RNA which is precipitated with isopropanol. The RNA pellet is washed with ethanol and redissolved in RNase-free water.

To remove any contaminating phenol, it is recommended to cleanup the isolated RNA using silica-membrane technology (such as RNeasy Kits, QIAGEN) or ethanol precipitation (see page 15). Phenol contamination can result in overestimation of RNA yield, inhibition in downstream applications, and decrease RNA stability.

Low amounts of genomic DNA may be present in the RNA. To avoid DNA-contamination affects in sensitive downstream applications, the RNA sample can be treated with DNase and cleaned up as described above.

Protocol 1: Lysis and homogenization of tissues using a rotor-stator homogenizator for isolation of RNA

This protocol is intended for fatty tissues, but can also be used with all other types of tissue.

Before starting

➔ Refer to supplier guidelines for instructions for operating the rotor–stator homogenizer.

➔ Be sure you are familiar with the section "Starting material guide" on page 16.

➔ Fresh, frozen, or stabilized tissues can be used. Flash-freeze in liquid nitrogen and immediately transfer to –70°C. Samples can be stored at this temperature for several months. Do not allow tissues to thaw before disruption in Isol-RNA Lysis Reagent.

➔ Homogenized tissue lysates can be stored at –70°C for at least 1 month. Before continuing with the protocol, incubate frozen lysates at 37°C in a water bath only until the sample is completely thawed and salts are dissolved.

Procedure

1. Add Isol-RNA Lysis Reagent to an appropriate vessel.

   This vessel will be used for disruption, homogenization, and subsequent centrifugation.

2. Add 1 ml Isol-RNA Lysis Reagent per 100 mg tissue is required.

   The volume of tissue should not exceed 10% of the volume of Isol-RNA Lysis Reagent.

   Generally, round-bottomed tubes allow more efficient disruption and homogenization than conical-bottomed tubes.
3. Remove the sample from storage or excise from the donor.

   RNA in harvested tissues is not protected until the tissues are flash-frozen, treated with RNAlater RNA Stabilization Reagent (QIAGEN), or disrupted and homogenized. Frozen tissues should not be allowed to thaw during handling. Work as quickly as possible.
4. Determine the amount of tissue and place it into the Isol-RNA Lysis Reagent. Proceed immediately to the next step immediately.

   Weighing tissue is the most accurate way to determine the amount.

   If the tissue sample was stored in RNAlater RNA Stabilization Reagent, remove it from the reagent using forceps and be sure to remove any crystals that may have formed.
5. Place the tip of the rotor–stator homogenizer disposable probe into the Isol-RNA Lysis Reagent.
6. Operate the rotor–stator homogenizer or at full speed until the tissue lysate is uniformly homogeneous (usually 20–40 s).

   Note: To avoid damage to the rotor–stator homogenizer and disposable probe during operation, make sure the tip of the probe remains submerged in the Isol-RNA Lysis Reagent.

   Note: Incomplete homogenization leads to significantly reduced RNA yields. Homogenization with the rotor–stator homogenizer or bead-mill generally results in higher RNA yields than with other methods.

   Optional: For samples containing a relatively high content of fat, proteins, polysaccharides, or extracellular material, centrifuge the homogenate at 12,000 x *g* for 10 min at 4°C to remove insoluble material. Carefully transfer the supernatant to a new tube, and proceed to step 4.
7. Place the tube containing the homogenate on the benchtop at room temperature (15–25°C) for 5 min.

   This step promotes dissociation of nucleoprotein complexes.
8. Add chloroform to the homoginate.

   Add 0.2 ml chloroform per 1 ml Isol-RNA Lysis Reagent.
9. Securely cap the tube containing the homogenate, and shake it vigorously for 15 s.

   Thorough mixing is important for subsequent phase separation.
10. Place the tube containing the homogenate on the benchtop at room temperature for 2–3 min.
11. Centrifuge at 12,000 x *g* for 15 min at 4°C.

After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase; and a lower, red, organic phase. For tissues with an especially high fat content, an additional clear phase may be visible below the red, organic phase. The volume of the aqueous phase is approximately 60% of the volume of the Isol-RNA Lysis Reagent pipetted in step 1.

12. Transfer the upper, aqueous phase to a new tube.
13. Add isopropanol, and mix thoroughly by vortexing.

    Add 0.5 ml isopropanol per 1 ml Isol-RNA Lysis Reagent.
14. Place the tube on the benchtop at room temperature for 10 min.
15. Centrifuge at 12,000 x *g* for 10 min at 4°C.
16. Carefully aspirate and discard the supernatant.

    The RNA pellet is often visible as a gel-like or white pellet at the bottom of the tube.
17. Add 75% ethanol.

    Add at least 1 ml of 75% ethanol per 1 ml Isol-RNA Lysis Reagent pipetted in step 1.
18. Centrifuge at 7500 x *g* for 5 min at 4°C.

    If the RNA pellet floats or sticks to the side of the tube, bring it to the bottom of the tube by centrifuging at 12,000 x *g* for 5 min at 4°C.
19. Remove the supernatant completely, and briefly air-dry the RNA pellet.

    Do not dry the RNA using a vacuum.
20. Redissolve the RNA in an appropriate volume of RNase-free water.

    We recommend using an RNeasy kit (QIAGEN) to clean up the RNA. Alternatively, the RNA can be cleaned up by an isopropanol precipitation (see page 15).

Protocol 2: Lysis and homogenization of tissues using a bead-mill for isolation of RNA

This protocol is intended for fatty tissues, but can also be used with all other types of tissue.

Before starting

➔ Refer to supplier guidelines for instructions for operating the bead-mill.

➔ Be sure you are familiar with the section "Starting material guide" on page 16.

➔ Fresh, frozen, or stabilized tissues can be used. Flash-freeze in liquid nitrogen and immediately transfer to –70°C. Samples can be stored at this temperature for several months. Do not allow tissues to thaw before disruption in Isol-RNA Lysis Reagent.

➔ Homogenized tissue lysates can be stored at –70°C for at least 1 month. Before continuing with the protocol, incubate frozen lysates at 37°C in a water bath only until the sample is completely thawed and salts are dissolved.

Procedure

1. Add the stainless steel beads in disruption tube according to the bead mill instructions.
2. Place the disruption tubes on dry ice.

   The tubes do not need to be placed on dry ice if the tissue samples are stabilized in RNAlater RNA Stabilization Reagent.
3. Remove the tissue sample from storage, or excise it from the donor.

   RNA in harvested tissues is not protected until the tissues are flash-frozen, treated with RNAlater RNA Stabilization Reagent (QIAGEN), or disrupted and homogenized. Frozen tissues should not be allowed to thaw during handling. The relevant procedures should be carried out as quickly as possible.
4. Determine the amount of tissue and place it into the Isol-RNA Lysis Reagent. Proceed immediately.

   Weighing tissue is the most accurate way to determine the amount.

   If the tissue samples were stored in RNAlater RNA Stabilization Reagent, remove them from the reagent using forceps and be sure to remove any crystals that may have formed.
5. Remove the tubes from the dry ice. Add Isol-RNA Lysis Reagent to each tube

   Add 1 ml Isol-RNA Lysis Reagent per 100 mg tissue is required. The volume of tissue should not exceed 10% of the volume of Isol-RNA Lysis Reagent.
6. Place the tubes in the adaptor set of the bead mill according to instructions.
7. Operate the bead mill for 2 min at 20 Hz.

8. Rearrange the tubes so that the outermost tubes are innermost and the innermost tubes are outermost.
9. Operate the bead mill for another 2 min at 20 Hz.

   The time and frequency depend on the tissue being processed and can be increased until the tissue is completely homogenized (e.g., up to 2 x 5 min at 25 Hz). Rearranging the tubes allows even homogenization. Do not reuse the stainless steel beads.

   Note: Incomplete homogenization leads to significantly reduced RNA yields. Homogenization with a bead-mill or rotor-stator homogenizer generally results in higher RNA yields than with other methods.

   Optional: For samples containing a relatively high content of fat, proteins, polysaccharides, or extracellular material, centrifuge the homogenate at 12,000 x *g* for 10 min at 4°C to remove insoluble material.

   Carefully transfer the supernatant to a new tube, and proceed to step 6.
10. Place the tubes containing the homogenates on the benchtop at room temperature (15–25°C) for 5 min.

    This step promotes dissociation of nucleoprotein complexes.
11. Add chloroform to the homoginate, and shake vigorously for 15 s.

    Add 0.2 ml chloroform per 1 ml Isol-RNA Lysis Reagent pipetted.

    Thorough mixing is important for subsequent phase separation.
12. Place the tubes containing the homogenates on the benchtop at room temperature for 2–3 min.
13. Centrifuge at 12,000 x *g* for 15 min at 4°C.

    After centrifugation, the sample separates into 3 phases: an upper, colorless, aqueous phase containing RNA; a white interphase; and a lower, red, organic phase. For tissues with an especially high fat content, an additional clear phase may be visible below the red, organic phase. The volume of the aqueous phase is approximately 60% of the volume of the Isol-RNA Lysis Reagent pipetted in step 3.
14. Transfer the upper, aqueous phase to a new tube. Add isopropanol, and mix thoroughly by vortexing.

    Add 0.5 ml isopropanol per 1 ml Isol-RNA Lysis Reagent.
15. Place the tubes on the benchtop at room temperature for 10 min.
16. Centrifuge at 12,000 x *g* for 10 min at 4°C.
17. Carefully aspirate and discard the supernatants.

    The RNA pellet is often visible as a gel-like or white pellet at the bottom of the tube.
18. Add 75% ethanol.

    Add at least 1 ml of 75% ethanol per 1 ml Isol-RNA Lysis Reagent.
19. Centrifuge at 7500 x *g* for 5 min at 4°C.

If the RNA pellet floats or sticks to the side of the tube, bring it to the bottom of the tube by centrifuging at 12,000 x *g* for 5 min at 4ºC.

20. Remove the supernatants completely, and briefly air-dry the RNA pellets.

    Do not dry the RNA using a vacuum.

21. Redissolve the RNA in an appropriate volume of RNase-free water.

    We recommend using an RNeasy kit (QIAGEN) to clean up the RNA. Alternatively, the RNA can be cleaned up by an isopropanol precipitation (see page 15).

Protocol 3: Isolation of genomic DNA and/or proteins from tissue samples treated with Isol-RNA Lysis Reagent

This protocol is used for the isolation of genomic DNA and/or protein from the interphase and organic (lower) phase of fatty tissue samples lysed in Isol-RNA Lysis Reagent.

Before starting

➔ All protocol steps should be performed at room temperature (15–25°C) unless otherwise stated.

➔ This protocol is scaled for lysates prepared with 1 ml Isol-RNA Lysis Reagent. Reagent volumes should be adjusted accordingly when other volumes of Isol-RNA Lysis Reagent have been used.

Two procedures are given below:

- **Procedure A: Isolation of genomic DNA from the organic phase of Isol-RNA Reagent-lysed fatty tissue samples**
- **Procedure B: Isolation of the protein fraction from the organic phase of Isol-RNA Reagent-treated fatty tissue samples**

Procedure A: Isolation of genomic DNA from the organic phase of Isol-RNA Reagent-lysed fatty tissue samples

1. Prepare samples according to protocols on page 7 or 12 until phase separation. Remove the upper aqueous phase completely, and set aside the interphase and the lower phases or store at 4°C overnight. Complete RNA isolation before continuing with the following protocol.
2. Remove any residues of the aqueous phase.

   This is critical for the quality of the isolated DNA.
3. Add 0.3 ml 100% ethanol to the interphase and phenol phase.
4. Carefully mix samples by inversion.
5. Incubate samples at room temperature (15–25°C) for 2–3 min.
6. Centrifuge at 2000 x *g* for 2 min at 4°C to sediment DNA.
7. Remove the phenol/ethanol supernatant and save for subsequent protein isolation.

Store the phenol/ethanol supernatant at 4°C, or start from step 7 in Procedure B to isolate protein immediately.

8. Add 1 ml sodium citrate solution to the DNA pellet.
9. Incubate at room temperature for 30 min.
10. Mixing by inversion every 5 min.
11. Centrifuge at 2000 x *g* for 5 min at 4°C.
12. Remove the supernatant.
13. Repeat steps 8 and 11 twice.

    After this wash step the DNA pellet can be stored in 75% ethanol at 4°C for over 3 months. For storage, remove the sodium citrate solution and add 2 ml 75% ethanol without redissolving the pellet.
14. Add 2 ml of 75% ethanol to the DNA pellet.
15. Incubate at room temperature for 20 min.
16. Mix by inversion every 5 min.
17. Centrifuge at 2000 x *g* for 5 min at 4°C.
18. Completely remove the ethanol supernatant.

    This ethanol wash removes pink color from the DNA pellet.
19. Air-dry the DNA pellet for 5–15 min.

    Do not dry using centrifugation, as the pellet will be more difficult to dissolve.
20. Redissolve the pellet in 8 mM NaOH to achieve the desired DNA concentration.

    Typically, addition of 300–600 µl of 8 mM NaOH to DNA isolated from 10^7 cells or 50–70 mg tissue will result in a DNA concentration of 0.2–0.3 µg/µl. Resuspension in a weak base is recommended since isolated genomic DNA does not resuspend well in water or Tris buffers.

    At this stage, the DNA preparation (especially from tissues) may contain insoluble gel-like material (e.g., fragments of membranes).
21. Centrifuge at 14,000 x *g* for 10 min at room temperature to remove insoluble material.
22. Transfer the supernatant to a new tube.

    The pH of 8 mM NaOH is approximately 9. For storage, the pH of the DNA sample solution should be adjusted to pH 7–8 by addition of TE or HEPES buffer.
23. To neutralize the DNA sample add 60 µl 0.1 M HEPES and 5.5 µl 100 mM EDTA (final concentration 1 mM) per 500 µl 8 mM NaOH used for redissolving the DNA pellet in step 21.

    Once the pH is adjusted, DNA can be stored long term at 4°C or –20°C.

Procedure B: Isolation of the protein fraction from the organic phase of Isol-RNA Reagent-treated fatty tissue samples

1. Prepare samples according to protocols on page 7 or 12 until phase separation. Remove the upper aqueous phase completely, and set aside the interphase and the lower phases or store at 4°C overnight. Complete RNA isolation before continuing with the following protocol.
2. Remove any residues of the aqueous phase.
3. Add 0.3 ml of 100% ethanol to the interphase and phenol phase.
4. Carefully mix samples by inversion.
5. Incubate samples at room temperature (15–25°C) for 2–3 min.
6. Centrifuge at 2000 x *g* at 4°C for 2 min to sediment DNA.
7. Transfer the phenol/ethanol supernatant containing the protein fraction to a new safe-lock reaction tube.

   The DNA pellet can be washed in sodium citrate and stored in 75% ethanol at 4°C for over 3 months (see Procedure B, step 8–13).
8. Add 1.5 ml isopropanol to precipitate the protein.
9. Mix by inversion for 15 s.
10. Incubate samples at room temperature (15–25°C) for 10 min.
11. Centrifuge at 12,000 x *g* for 10 min at 4°C.
12. Remove the supernatant.
13. Add 2 ml guanidine–ethanol solution to the pellet containing the protein.
14. Incubate at room temperature for 20 min.

    The protein pellet can be stored in guanidine-ethanol solution at 4°C (for at least 1 month) or –20°C (for at least 1 year).
15. Centrifuge at 7500 x *g* for 5 min at room temperature.
16. Remove the supernatant.
17. Repeat steps 13 and 15 twice.
18. Add 2 ml of 100% ethanol to the pellet containing the proteins and vortex.
19. Incubate at room temperature for 20 min.
20. Centrifuge at 7500 x *g* for 5 min at room temperature.
21. Remove the supernatant.
22. Air-dry the pellet for 5–10 min.

    Do not dry under centrifugation, as the pellet will be more difficult to dissolve.
23. Add 50 µl urea/DTT solution, and break up the pellet using a needle.
24. Add 450 µl urea/DTT solution, and incubate at room temperature for 1 h.
25. Incubate at 95°C for 3 min then place the tube on ice. During the incubation on ice sonicate 10 times using short bursts.

    All proteins should be in solution. If not, repeat step 17 once or twice. Sonication should be performed on ice to avoid foaming of the sample.
26. Centrifuge at 10,000 x *g* for 10 min at room temperature.

27. Transfer the supernatant containing the proteins to a new tube.

    Proteins dissolved in urea/DTT solution can be directly analyzed by SDS-PAGE/Western blot or protein assays such as Bradford, or stored at 4°C overnight or at –20°C for at least 1 year.

Supporting information

Additional protocol: RNA cleanup with isopropanol precipitation

1. Adjust the salt concentration if necessary.

   Sodium acetate (0.3 M, pH 5.2, final concentration) or ammonium acetate (2.0–2.5 M, final concentration) can be used.
2. Add 0.6–0.7 volumes of room-temperature isopropanol to the DNA solution and mix well.

   Use all solutions at room temperature to minimize co-precipitation of salt.
3. Centrifuge the sample immediately at 10,000–15,000 x *g* for 15–30 min at 4°C.

   Centrifugation should be carried out at 4°C to prevent overheating of the sample. (When precipitating from small volumes, centrifugation may be carried out at room temperature.)
4. Carefully decant the supernatant without disturbing the pellet.

   Marking the outside of the tube or uniformly orienting microcentrifuge tubes before centrifugation allows the pellet to be more easily located. Pellets from isopropanol precipitation have a glassy appearance- and may be more difficult to see than the fluffy salt-containing pellets that result from ethanol precipitation.

   Care should be taken when removing the supernatant as pellets from isopropanol precipitation are more loosely attached to the side of the tube.

   Carefully tip the tube with the pellet on the upper side to avoid dislodging the pellet.

   For valuable samples, the supernatant should be retained until recovery of the precipitated DNA has been verified.
5. Wash the DNA pellet by adding room-temperature 70% ethanol. This removes co-precipitated salt and replaces the isopropanol with the more volatile ethanol, making the DNA easier to redissolve.

Starting material guide

Handling and storing starting material

RNA in tissues can be protected with immediate treatment with one of the following:

- Flash freezing
- Treating with a stabilization reagent, such as RNAlater RNA Stabilization Reagent (QIAGEN)
- Disrupting and homogenizing in the presence of RNase-inhibiting or denaturing reagents

If the tissue is not treated immediately, the gene expression profile might occur.

Frozen tissue samples should not be allowed to thaw until disruption.

Samples can be stored at –70°C for at least 1 month after homogenization in Isol-RNA Lysis Reagent.

Creating a lysate

Complete disruption and homogenization is required for maximal RNA yields.

To release all the RNA contained in the sample, plasma membranes of cells and organelles must be disrupted.

To reduce the viscosity of the lysates produced by disruption, the samples must be homogenized to shear high-molecular-weight genomic DNA and other cellular components.

Rotor-stator homogenizers

The blade of the rotor–stator probe rotates at a very high speed, causing the sample to be disrupted and homogenized by a combination of turbulence and mechanical shearing. Depending on the sample, individual tissue samples can be thoroughly disrupted and simultaneously homogenized in the presence of lysis buffer using rotor–stator homogenizer in 15–90 seconds.

Bead-mills

The beads in a bead mill are agitated to disrupt and homogenise cells in the presence of lysis buffer. Depending on the adapter used, a bead-mill can process up to 192 samples simultaneously.

Note: Tungsten carbide beads should not be used as they react with Isol-RNA Lysis Reagent.

Troubleshooting

Observation:	**Phases do not separate completely**
Possible cause:	No chloroform added or chloroform not pure
Avoiding:	Make sure to add chloroform that does not contain isoamyl alcohol or other additives.
Possible cause:	Homogenate not sufficiently mixed before centrifugation
Avoiding:	After addition of chloroform, the homogenate must be vigorously shaken. If the phases are not well separated, shake the tube vigorously for at least 15 s, and repeat subsequent incubation and centrifugation.
Possible cause:	Organic solvents in samples used for RNA purification
Avoiding:	Make sure that the starting sample does not contain organic solvents (e.g., ethanol, DMSO), strong buffers, or alkaline reagents. These can interfere with the phase separation.
Observation:	**RNA difficult to dissolve**
Possible cause:	RNA pellet overdried
Avoiding:	Air-dry RNA pellets instead of using a vacuum. If necessary, dissolve the RNA in a larger volume of RNase-free water, or allow more time for the RNA to dissolve.
Possible cause:	Too much isopropanol in RNA pellet
Avoiding:	Be sure to wash the RNA pellet with 75% ethanol, as described in the protocol, to remove isopropanol. If necessary, dissolve the RNA in a larger volume of RNase-free water, or allow more time for the RNA to dissolve.

Observation:	**Low RNA yield**
Possible cause:	Insufficient disruption and homogenization
Avoiding:	See page **Error! Bookmark not defined.** for details on disruption and homogenization methods. In subsequent preparations, reduce the amount of starting material and/or increase the volume of Isol-RNA Lysis Reagent and the homogenization time.
Possible cause:	RNA pellet incompletely dissolved
Avoiding:	Check for residual pellet. Be sure to wash any RNA from the side of the tube, especially if the tube is made of glass. See also "RNA difficult to dissolve" above.

Observation:	**Low A_{260}/A_{280} value**
Possible cause:	Not enough Isol-RNA Lysis Reagent used for homogenization
Avoiding:	In subsequent preparations, reduce the amount of starting material and/or increase the volume of Isol-RNA Lysis Reagent and the homogenization time.
Possible cause:	Contamination of aqueous phase with phenol
Avoiding:	When removing the aqueous phase, be sure not to carry over any of the other phases. After the Isol-RNA procedure, clean up the RNA by following an RNeasy RNA cleanup protocol.
Possible cause:	Sample not incubated for 5 min after homogenization
Avoiding:	Place the sample at room temperature (15–25°C) for 5 min after homogenization, as indicated in the protocol. This step is important to promote dissociation of nucleoprotein complexes.

Possible cause:	RNA pellet incompletely dissolved
Avoiding:	Check for residual pellet. Be sure to wash any RNA from the side of the tube, especially if the tube is made of glass. See also "RNA difficult to dissolve" above.

Possible cause:	Water used to dilute RNA for A_{260}/A_{280} measurement
Avoiding:	Use 10 mM Tris-Cl, pH 7.5, not RNase-free water, to dilute the sample before measuring purity.

Observation:	**RNA degraded**
Possible cause:	Inappropriate handling of starting material
Avoiding:	For frozen tissue samples, ensure that they were flash-frozen immediately in liquid nitrogen and properly stored at –70°C. Perform the Isol-RNA procedure quickly, especially the first few steps., see page 15.

Observation:	**DNA contamination in downstream experiments**
Possible cause:	Phase separation performed at too high a temperature
Avoiding:	The phase separation should be performed at 4°C. Make sure that the centrifuge does not heat above 10°C during the centrifugation.

Possible cause:	Interphase contamination of aqueous phase
Avoiding:	Contamination of the aqueous phase with the interphase results in an increased DNA content in the purified RNA. Make sure to transfer the aqueous phase without interphase contamination.

Possible cause:	Not enough Isol-RNA Lysis Reagent used for homogenization
Avoiding:	In subsequent preparations, reduce the amount of starting material and/or increase the volume of Isol-RNA Lysis Reagent and the homogenization time.

Possible cause:	No DNase treatment
Avoiding:	Treat the RNA sample with DNase and then clean up the RNA using an RNeasy Kit. Alternatively, carry out RNA cleanup and on-column DNase digestion using an RNeasy Kit. For details, see the handbook supplied with the RNeasy Kit.

Ordering information

Product	Size	Order/ref. no.
Isol-RNA Lysis Reagent	200 ml	2302700
RNAse free DNase Set	50 reactions	2500120
Masterscript Kit	50 reactions	2201000
Masterscript Kit	200 reactions	2201010
Masterscript RT-PCR System	20 reactions	2201100
Masterscript RT-PCR System	100 reactions	2201110
Phase Lock Gel Light 1.5 ml	200 tubes	2302800
Phase Lock Gel Light 2 ml	200 tubes	2302820
Phase Lock Gel Light 15 ml	100 tubes	2302840
Phase Lock Gel Light 50 ml	25 tubes	2302860
Phase Lock Gel Heavy 1.5 ml	200 tubes	2302810
Phase Lock Gel Heavy 2 ml	200 tubes	2302830
Phase Lock Gel Heavy 15 ml	100 tubes	2302850
Phase Lock Gel Heavy 50 ml	25 tubes	2302870
Water, Mol Bio grade	1 l	2500000
Water, Mol Bio grade	10 x 50 ml	2500010
Water, Mol Bio grade	5 l	2500020
DNA Gel Loading Buffer 10x	6 x 500 µl	2500070
TBE 5x	5 l	2500050
TAE 50x	5 l	2500060

5 PRIME distributors

A complete list of 5 PRIME distributors is available from www.5PRIME.com.