ZYMO RESEARCH

RNA Purification Made Simple™

# Direct-zol™ RNA Microprep

TRIzol® In. RNA Out.

## Highlights

- 7-minute, spin-column purification of total RNA (including small/microRNAs) ***directly*** from TRIzol®, TRI Reagent® or similar acid-guanidinium-phenol based reagents.
- Extract total RNA from low inputs (down to a single cell).
- No need for chloroform, phase-separation or precipitation steps.
- RNA is ready for Next-Gen Sequencing, RT-qPCR, etc. *DNase I is included.*

Catalog Numbers:
R2060, R2061, R2062, R2063

Scan with your smart-phone camera to view the online protocol/video.

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# Table of Contents

# Product Contents

| Direct-zol™ RNA Microprep | R2060 (50 prep) | R2061 (50 prep) | R2062 (200 prep) | R2063 (200 prep) |
|---|---|---|---|---|
| TRI Reagent® | - | 50 ml | - | 200 ml |
| Direct-zol™ RNA PreWash[1] (concentrate) | 40 ml | 40 ml | 160 ml | 160 ml |
| RNA Wash Buffer[2] (concentrate) | 12 ml | 12 ml | 48 ml | 48 ml |
| DNase I[3] (lyophilized) | 1500 U | 1500 U | 1500 U (x4) | 1500 U (x4) |
| DNA Digestion Buffer | 4 ml | 4 ml | 16 ml | 16 ml |
| DNase/RNase-Free Water | 6 ml | 6 ml | 30 ml | 30 ml |
| Zymo-Spin™ IC Columns | 50 pcs | 50 pcs | 200 pcs | 200 pcs |
| Collection Tubes | 100 pcs | 100 pcs | 400 pcs | 400 pcs |
| Instruction Manual | 1 pc | 1 pc | 1 pc | 1 pc |

**Storage Temperature** - Store all kit components (i.e., buffers, columns) at room temperature.
Before use:

1 Add 10 ml or 40 ml ethanol (95-100%) to the 40 ml or 160 ml **Direct-zol™ RNA PreWash** concentrate, respectively.

2 Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate.

3 Reconstitute lyophilized **DNase I** with **DNase/RNase-Free Water**, mix by gentle inversion and store frozen aliquots:
**#E1011-A (1500 U)**, add 275 µl **water**
#E1009-A (250 U), add 55 µl water

# Specifications

- **Sample Sources –** Any sample stored and preserved in TRIzol®, TRI Reagent® or similar[1]. (animal cells, tissue, bacteria, yeast, biological fluids, samples stored in DNA/RNA Shield™ and in-vitro processed RNA (e.g., transcription products, DNase-treated or labeled RNA)).

- **Sample Inactivation –** TRI Reagent® (provided with **R2061**, **R2063** only) inhibits RNase activity and inactivates viruses and other infectious agents.

- **Size –** Total RNA including small/microRNAs (≥ 17 nt).

- **Purity –** $A_{260}/A_{280}$ & $A_{260}/A_{230}$ > 1.8. RNA is ready for Next-Gen Sequencing, RT-qPCR, etc.

- **Binding Capacity –** 10 µg total RNA (**Zymo-Spin™ IC Column**).

- **Compatibility –** TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ or similar acid-guanidinium-phenol based reagents can be used in place of TRI Reagent®.

  Also, compatible with samples in TRIzol®, TRI Reagent® or similar reagent that contain chloroform, 1-bromo-3-chloropropane (BCP), or 4-bromoanisole (BAN), the aqueous phase of phase-separated samples and samples stored in RNAlater™ (page 8). For compatibility with cetyltrimethylammonium bromide (CTAB)-based extraction, see detailed protocol here.

- **Elution Volume –** ≥ 6 µl **DNase/RNase-Free Water**.

- **Equipment Needed** (user provided) **–** Microcentrifuge.

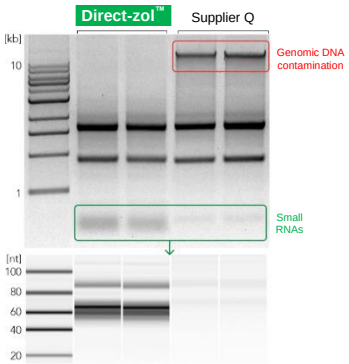
1 RNAzol®, QIAzol®, TriPure™, TriSure™ or similar acid-guanidinium-phenol reagent.

# Product Description

The **Direct-zol™ RNA Microprep** provides a streamlined method for the purification of up to 10 µg (per prep) of high-quality RNA directly from low input samples (down to a single cell) in TRIzol®, TRI Reagent® or similar[1]. Total RNA including small RNAs (17-200 nt)[2], is effectively isolated from a variety of sample sources (cells, tissues, serum, plasma, blood, samples stored in DNA/RNA Shield™, etc.).


Simply add ethanol to a TRI Reagent® sample, bind directly to the **Zymo-Spin™ Column**, wash, and elute RNA. No phase separation, precipitation, or post-purification steps are necessary. RNA is high-quality and ready for Next-Gen Sequencing, RT-qPCR, transcription profiling, hybridization, etc.

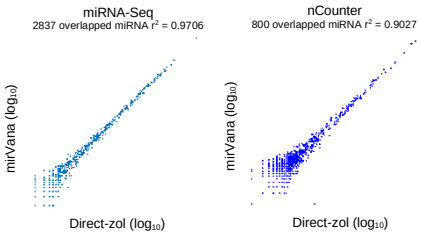
## Efficient recovery of DNA-free Total RNA




(top) High-quality DNA-free RNA is purified from human epithelial cells using the **Direct-zol™** procedure compared to a preparation from Supplier Q (1% agarose/TAE gel).

(bottom) Small RNAs are efficiently recovered with the **Direct-zol™** procedure. However, this is not the case with Supplier Q's prep (Bioanalyzer, Small RNA Chip).

## Complete miRNA recovery




MicroRNA isolation using **Direct-zol™ RNA** kits**.** The data show RNA purified from TRIzol® samples using the Direct-zol™ RNA MiniPrep compared to a method known to be unbiased[2] (mirVana™, Ambion).
MicroRNA analysis was performed using miRNA-seq (MiSeq®, Illumina) and direct hybridization assay (nCounter®, Nanostring).

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1 RNAzol®, QIAzol®, TriPure™, TriSure™ or similar acid-guanidinium-phenol reagent.
2 RNA isolation by conventional phase separation was shown to selectively enrich for some species of miRNA, leading to bias in downstream analysis (Kim et al. 2012. Molecular Cell 46(6):893-895). Direct-zol™ RNA method assures complete recovery of small/miRNAs.

## Input Capacity and Average RNA Yield

| Input | Average RNA Yield | Kit Capacity |
|---|---|---|
| **Cells** | 1 µg (per $10^5$ cells) | Up to $10^6$ |
| HeLa | 1.5 µg | |
| **High Yield Tissue**[1] (mouse) | ≥ 3 µg (per 1 mg) | Up to 2 mg |
| Spleen | 3-5 µg | |
| Liver | 4-6 µg | |
| **Low Yield Tissue**[1] (mouse) | ≤ 3 µg (per 1 mg) | Up to 5 mg |
| Brain, Heart | 0.5-1.5 µg | |
| Muscle | 0.5-2 µg | |
| Lung | 1-2 µg | |
| Intestine | 1-3 µg | |
| Kidney | 2-3 µg | |
| **Whole Blood**[2] | (per 100 µl) | Up to 200 µl |
| Porcine | 1-2 µg | |
| Human | 0.2-1 µg | |

1 Yield from tissue can vary due to other factors (i.e., organism type, physiological state, and growth conditions.
2 Yield from blood can vary based upon collection, sample preparation, donor, age, and/or health conditions.

# Protocol

The protocol consists of: (I) Buffer Preparation, (II) Sample Preparation and (III) RNA Purification.

The following guidelines are provided for processing various sample types in TRIzol®, TRI Reagent® or similar[1] acid-guanidinium-phenol reagents prior to column purification of the RNA (see page 4 for Input Capacity and Average RNA Yield).

## (I) Buffer Preparation

- ✓ Add 10 ml or 40 ml ethanol (95-100%) to the 40 ml or 160 ml **Direct-zol™ RNA PreWash** concentrate, respectively.
- ✓ Add 48 ml 100% ethanol (52 ml 95% ethanol) to the 12 ml **RNA Wash Buffer** concentrate or 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **RNA Wash Buffer** concentrate.
- ✓ Reconstitute lyophilized **DNase I** with **DNase/RNase-Free Water**, mix by gentle inversion and store frozen aliquots:
  **#E1011-A (1500 U)**, add 275 µl **water**
  #E1009-A (250 U), add 55 µl water

## (II) Sample Preparation[2]

- ✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 1 minute.

### Cells

Lyse animal or gram(-) bacteria cells* directly in a culture dish** or resuspend pelleted cells in an appropriate volume (see table below) of TRI Reagent® or similar[1] and mix thoroughly. Proceed to RNA Purification (page 7).

| Animal | Gram(-) bacteria | Add TRI Reagent® |
|---|---|---|
| ≤ $10^5$ | - | ≥ 100 µl |
| ≤ $10^6$ | ≤ $10^8$ | ≥ 300 µl |

* For cell suspensions, add 3 volumes of TRI Reagent® to 1 volume of cell suspension.
** For direct lysis in a dish, add 100 µl for each $cm^2$ of culture surface area.

1 TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ or similar acid-guanidinium-phenol reagent.
2 RNA yield can vary with sample types, organism, quality and treatment of the starting material (see page 4). To ensure complete lysis and homogenization of a sample, use a sufficient amount of TRIzol®, TRI Reagent® or similar reagent. For detailed processing information, refer to the TRI Reagent® product manual (or manufacturer's instructions for the reagent used).

## Tough-to-Lyse Samples

Tough-to-lyse samples (see table below) can be homogenized in ≥ 800 µl TRIzol®, TRI Reagent® or similar[1] with a mortar/pestle, dounce, syringe, tissue grinder, or bead beating with a high-speed homogenizer.

To remove particulate debris from homogenized tissue, centrifuge and transfer the supernatant into a new nuclease-free tube. Proceed to RNA Purification (page 7).

Recommended: Use ZR BashingBead™ Lysis Tubes (materials sold separately; #S6012, #S6003, #S014) for complete lysis and homogenization.

| Input | Gram(-) bacteria (optional; easy-to-lyse) | Gram(+) bacteria | Tissue (animal, plant*) | Pathogen (microbes in tissue) |
|---|---|---|---|---|
| per prep | bacteria (≤ $10^8$) | bacteria (≤ $10^8$)<br>yeast (≤ $10^7$) | animal: high yield (≤ 2 mg)<br>animal: low yield (≤ 5 mg)<br>plant* (≤ 20 mg) | animal/insect, plant (≤ 5 mg) |
| lysis beads catalog # | 0.5 mm and 0.1 mm; **S6012** | 0.5 mm and 0.1 mm; **S6012** | 2.0 mm; **S6003** | 2.0 mm and 0.1 mm; **S6014** |
| high-speed[2,3] | 30 sec | 5-10 min | 30-60 sec | 3-5 min |
| low-speed[3] | 5-10 min | 20-40 min | 3-5 min | 5-10 min |

*Compatible with CTAB-based RNA extraction methods for polysaccharide-rich and/or phenolics-rich samples (e.g., Pinus, Geranium plants). Please find detailed protocol here.

## Liquids

Add an appropriate volume of TRI Reagent® or similar[1] to a liquid sample and mix thoroughly (see table below). To remove particulate debris (if any), centrifuge and transfer the supernatant into an RNase-free tube. Proceed to RNA Purification (page 7).

Recommended: For biological samples (whole-blood, plasma, serum, buffy coat, PBMCs, WBCs, FACS, etc.) or samples collected in DNA/RNA Shield™[4], perform Proteinase K treatment[5] (sold separately) prior to adding TRI Reagent®.

| Sample | | Add TRI Reagent® |
|---|---|---|
| **Biological liquid** (blood, plasma, serum, WBCs, FACs, etc.) or **Reaction clean-up** (DNase I treated RNA, *in vitro* transcription, labeling, etc.). | 100 µl | ≥ 300 µl |
| **Samples in DNA/RNA Shield™** (biological sample[4,5] or stored purified RNA). | 100 µl | 100 µl |

1 TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ or similar acid-guanidinium-phenol reagent.
2 Perform high-speed homogenization at 1-minute intervals (including a cooling step for 3-5 minutes), to avoid overheating the machine and/or breaking the tube.
3 High-speed homogenizers (e.g., MP Bio FastPrep-24™, Bertin Precellys, etc.). Low-speed homogenizers (e.g., Vortex Genie, etc.).
4 DNA/RNA Shield™ reagent (R1100, R1200) or DNA/RNA Shield™ Blood Collection Tube (R1150).
5 For Proteinase K treatment, see page 9.

## (III) RNA Purification

✓ Perform all steps at room temperature and centrifugation at 10,000-16,000 x g for 30 seconds, unless specified.

1. Add an equal volume ethanol (95-100%) to a sample lysed in TRI Reagent® or similar[1] and mix thoroughly.

   Example: Add 400 µl ethanol to 400 µl mixture (sample lysed in TRI Reagent®).

2. Transfer the mixture into a **Zymo-Spin™ IC Column**[2] in a **Collection Tube** and centrifuge[3]. Transfer the column into a **new** collection tube and discard the flow-through.

3. **DNase I**[4] treatment (recommended)

   (D1) Add 400 µl **RNA Wash Buffer** to the column and centrifuge.

   (D2) In an RNase-free tube (not included), add 5 µl **DNase I** (6 U/µl)*, 35 µl **DNA Digestion Buffer** and mix by gentle inversion. Add the mix directly to the column matrix.

   (D3) Incubate at room temperature (20-30°C) for 15 minutes. Proceed to step 4.

4. Add 400 µl **Direct-zol™ RNA PreWash**[5] to the column and centrifuge. Discard the flow-through and repeat this step.

5. Add 700 µl **RNA Wash Buffer**[5] to the column and centrifuge for 1 minute to ensure complete removal of the wash buffer. Transfer the column carefully into an RNase-free tube (not included).

6. To elute RNA, add 15 µl of **DNase/RNase-Free Water** directly to the column matrix and centrifuge.

   Alternatively, for highly concentrated RNA use ≥ 6 µl elution.

   The eluted RNA[6] can be used immediately or stored frozen.

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1 TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ or similar acid-guanidinium-phenol reagent.
2 To process samples > 700 µl, reload the column and repeat Step 2.
3 At this point, proteins can be purified from the flow-through (see page 9).
4 Prior to use, reconstitute the lyophilized **DNase I** (Buffer Preparation, page 5). * Unit definition – one unit increases the absorbance of a high molecular weight DNA solution at a rate of 0.001 $A_{260}$ units/ml of reaction mixture at 25°C.
5 Before use, add ethanol to the buffer concentrate (Buffer Preparation, page 5).
6 For complete removal of PCR (RT) inhibitors from plant, soil and fecal samples, use the OneStep™ PCR Inhibitor Removal Kit (D6030).

# Appendices

## RNA purification from aqueous phase after TRI Reagent® extraction

For samples that have already been phase separated in TRI Reagent®[1] or similar[2], simply transfer the aqueous phase[3] containing RNA into an RNase-free tube. Add an equal volume ethanol (95-100%) to the aqueous phase (1:1) and mix thoroughly. Proceed to RNA Purification (page 7, step 2).

## RNA extraction from samples stored in RNAlater™

Cells

Pellet cells[4] at up to 5,000 x g and remove the RNAlater™ (supernatant) prior to RNA extraction. Then lyse the cell pellet in TRI Reagent® (Sample Preparation, Cells, page 5).

Note: To extract RNA from cells without reagent removal, use 10 volumes of TRI Reagent® per sample volume. Proceed to phase separation and process the aqueous phase. Simply transfer the aqueous phase containing RNA into an RNase-free tube. Then add an equal volume ethanol (95-100%) to the aqueous phase (1:1) and mix thoroughly. Proceed to RNA Purification (page 7, step 2).

Tissue

Remove tissue from RNAlater™ using forceps. Eliminate any excess reagent or crystals that may have formed and proceed immediately with extraction in TRI Reagent® (Sample Preparation, Tough-to-lyse samples, page 6).

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1 For detailed processing information, refer to the TRI-Reagent® product manual (or manufacturer's instructions for the reagent used).
2 TRIzol®, RNAzol®, QIAzol®, TriPure™, TriSure™ or similar acid-guanidinium-phenol reagents.
3 Alternatively, the aqueous phase can be processed with the RNA Clean & Concentrator™ (R1015).
4 Different cells may react differently to centrifugation forces and it is recommended to test the pelleting procedure with non-valuable samples first. Diluting RNAlater™ by 50% with cold PBS reduces solution density allowing for lower forces during cell pelleting (e.g., 500 x g).

## Protein Purification

The protein content (denatured) in the flow-through after the RNA binding to the column can be purified (see RNA Purification, page 7, step 2):

1. Add 4 volumes of cold acetone (-20°C) to the flow-through (4:1) and mix.
2. Incubate the samples for 30 minutes on ice.
3. Centrifuge at max speed for 10 minutes. Discard the supernatant. Keep the pellet.
4. Add 400 µl ethanol (95-100%) to the protein pellet. Centrifuge at max speed for 1 minute. Discard the supernatant.
5. Air-dry the protein pellet for 10 minutes at room temperature.
6. Resuspend and vortex the pellet in a buffer appropriate for downstream application (e.g., SDS-PAGE sample loading buffer).

## Proteinase K Treatment

- ✓ Proteinase K treatment can be performed on protein-rich samples stored in **DNA/RNA Shield™** (2X concentrate; #R1200) (e.g., tissue, blood cells, plasma, serum, saliva, sputum, etc.) using **Proteinase K Set** (#D3001-2-5, D3001-2-20; sold separately).

Add 10 µl Proteinase K (reconstituted) to 1 ml DNA/RNA Shield sample (scale proportionally) and mix by inversion. Then incubate at room temperature (20-30°C) for 30 minutes (homogenized) or 2-5 hours (non-homogenized). Optimization may be required.

## Compatibility with CTAB-Based Methods

The Direct-zol RNA kits are compatible with CTAB-based RNA extraction methods for polysaccharide-rich and/or phenolics-rich samples (e.g., Pinus, Geranium plants). Please find detailed protocol here.

1 Yield from tissue can vary due to other factors (i.e., organism type, physiological state, and growth conditions.
2 Yield from blood can vary based upon collection, sample preparation, donor, age, and/or health conditions.

# Ordering Information

| Product Description | Catalog No. | Size |
|---|---|---|
| **Direct-zol™ RNA Microprep**<br>(TRI Reagent® not included) | R2060<br>R2062 | 50 preps.<br>200 preps. |
| **Direct-zol™ RNA Microprep**<br>(supplied with TRI Reagent®) | R2061<br>R2063 | 50 preps.<br>200 preps. |

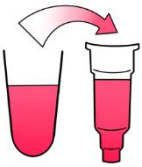
| Individual Kit Components | Catalog No. | Amount |
|---|---|---|
| **TRI Reagent®** | R2050-1-50<br>R2050-1-200 | 50 ml<br>200 ml |
| **Direct-zol™ RNA PreWash** (concentrate) | R2050-2-40<br>R2050-2-160 | 40 ml<br>160 ml |
| **RNA Wash Buffer** (concentrate) | R1003-3-6<br>R1003-3-12<br>R1003-3-24<br>R1003-3-48 | 6 ml<br>12 ml<br>24 ml<br>48 ml |
| **Zymo-Spin™ IC Columns** | C1004-50 | 50 |
| **Collection Tubes** | C1001-50<br>C1001-500<br>C1001-1000 | 50<br>500<br>1000 |
| **DNase/RNase-Free Water** | W1001-1<br>W1001-4<br>W1001-6<br>W1001-10<br>W1001-30 | 1 ml<br>4 ml<br>6 ml<br>10 ml<br>30 ml |
| **DNase I** (lyophilized)<br>(Supplied with DNA Digestion Buffer, 4 ml) | E1010<br>E1011 | 1 set (250 U)<br>1 set (1500 U) |
| **DNA/RNA Shield™** (2X concentrate) | R1200-25<br>R1200-125 | 25 ml<br>125 ml |
| **Proteinase K Set** (w/ Storage Buffer) | D3001-2-5<br>D3001-2-20 | 5 mg<br>20 mg |

# Complete Your Workflow

✓ For tough-to-lyse samples in TRIzol, use ZR BashingBead Lysis Tubes:

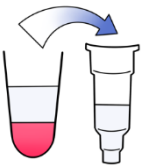
| ZR BashingBead Lysis Tubes | |
|---|---|
| 2.0 mm beads #S6003 | For plant/animal tissue |
| 0.1 + 0.5 mm beads #S6012 | For microbes |
| 0.1 + 2.0 mm beads #S6014 | For microbes in tissue/insects |

✓ The only **direct**, high-throughput and automatable RNA purification from sample lysates in TRIzol (DNase I Set included with all formats):



| Direct-zol RNA kits | |
|---|---|
| Microprep #R2060-R2063 | From 1 cell and up |
| Miniprep #R2050-R2053 | Up to 50 ug RNA |
| Miniprep Plus #R2070-R2073 | Up to 100 ug RNA |
| 96-well #R2054-R2057 | Spin-plate |
| MagBeads #R2100-R2105 | Automatable (Tecan, Hamilton, Kingfisher, etc.) |

✓ For RNA clean-up (purification) from the aqueous phase (e.g., TRIzol, TRI Reagent or similar) or from any enzymatic reaction (e.g., DNase I treated RNA):

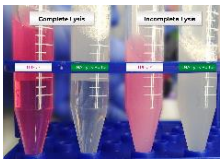


| RNA Clean & Concentrator kit | |
|---|---|
| #R1013-R1014 | DNase I Set included |

✓ For NGS:

| Zymo-Seq RiboFree Total RNA Library Prep kit | |
|---|---|
| #R3000 | 12 preps |
| #R3003 | 96 preps |

# Troubleshooting Guide

| Problem | Possible Causes and Suggested Solutions |
|---|---|
| **Precipitation, viscous lysate** | Incomplete lysis and/or high-mass input:<br>- If precipitation occurs (upon adding ethanol to the lysate) or if the lysate is extremely viscous, increase the volume of TRIzol®, TRI Reagent® or similar reagent to ensure complete lysis and homogenization until lysate is transparent (see image).<br>  |
| **Low purity** ($A_{260}/A_{230}$ nm, $A_{260}/A_{280}$ nm) | Sample handling:<br>- Ethanol and/or salt contamination. After centrifugation steps, carefully remove the column from the collection tube to prevent buffer carryover. Alternatively, blot emptied collection tube with a tissue or towel.<br>- Make sure lysate and wash buffers have passed completely through the matrix of the column. This may require centrifuging at a higher speed and/or longer time.<br>Incomplete lysis and/or cellular debris:<br>- Increase the volume of TRIzol®, TRI Reagent® or similar to ensure complete lysis and homogenization. Be sure to centrifuge any cellular debris and then process the cleared lysate. |
| **Low yield** | Sample input:<br>- Too much input or incomplete lysis/homogenization can cause cellular debris to clog or overload the column and result in compromised RNA recovery. Use less input material and/or increase TRIzol®, TRI Reagent® or similar reagent.<br>High-protein content (blood, plasma/serum, etc.)<br>- Perform Proteinase K treatment to the sample prior to adding TRIzol®, TRI Reagent® or similar reagent (Sample preparation, Liquids, page 6). |
| **DNA contamination** | To remove DNA:<br>- Perform in-column DNase I treatment (page 7) or perform DNase I treatment post-purification (R1013, page 4), then re-purify the treated sample.<br>- For future preps, increase the volume of TRIzol®, TRI Reagent® or similar reagent to ensure complete lysis and homogenization of the sample. |
| **RNA degradation** | To prevent RNA degradation:<br>- Immediately collect and lyse fresh sample into TRIzol®, TRI Reagent® or similar reagent to ensure RNA stability. Homogenized samples in TRIzol®, TRI Reagent® or similar can be stored frozen for later processing. |

For technical assistance, please contact 1-888-882-9682 or email tech@zymoresearch.com

# Notes

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Reagents are routinely tested on a lot-to-lot basis to ensure they provide the highest performance and reliability.

This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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