Organic Extraction of RNA From Animal and Plant Tissues Without Phase Separation

ZYMO RESEARCH
The Beauty of Science is to Make Things Simple

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ABSTRACT

Organic acid-guanidinium-phenol based extraction is widely implemented for most plant and animal RNA purification protocols. This organic extraction method effectively inactivates nucleases and infectious agents and allows for adequate sample storage and stabilization. However, limitations of existing organic extraction methods become apparent with high-throughput processing and the handling of small volume inputs. In both cases, the requirement of phase separation can affect both the yield and purity of RNA. Here we present an alternative acid-guanidinium-phenol based procedure that effectively bypasses phase separation/precipitation steps with a spin column and specially designed washes. This helps to eliminate problems attributed to phenol carryover that is often associated with conventional organic extraction methods. This new "Direct-zol™" procedure maximizes total RNA recovery, including small RNAs, without the need for a carrier. For tough-to-lyse plant and animal tissue the Direct-zol™ procedure can be combined with unique *BashingBead™* and *OneStep* inhibitor removal technologies for unparalleled yields and complete removal of polyphenolic inhibitors to RT-PCR, respectively. The Direct-zol™ procedure delivers DNA-free RNA that is ideal for RT-PCR, RNA-seq (expression profiling), hybridization, etc.

INTRODUCTION

Acid guanidinium-phenol based reagents for RNA isolation [1,2,3] have been introduced 25 years ago and are still amongst the most popular methods for isolating RNA. TRI-Reagent® *(TRIzol®, etc)* is exceptional in providing RNA stabilization and inactivation of infectious agents. However, phase separation, precipitation, and potential phenol carryover pose challenges for RNA (including small RNA) recovery. This has been resolved by development of the Direct-zol™ RNA purification procedure that enables use of Trizol® (and similar) without phase separation and precipitation. Instead, novel spin column technology is used for isolation of ultra-pure broad range RNA directly from samples in TRI-Reagent® or similar.

OVERVIEW

Direct-zol™ RNA

- ✓ *For samples in TRI-Reagent® or similar*
- ✓ *No phase separation*
- ✓ *No precipitation*
- ✓ *No phenol carryover*
- ✓ *Broad range RNA recovery*
- ✓ *DNA-free RNA*

Direct Workflow

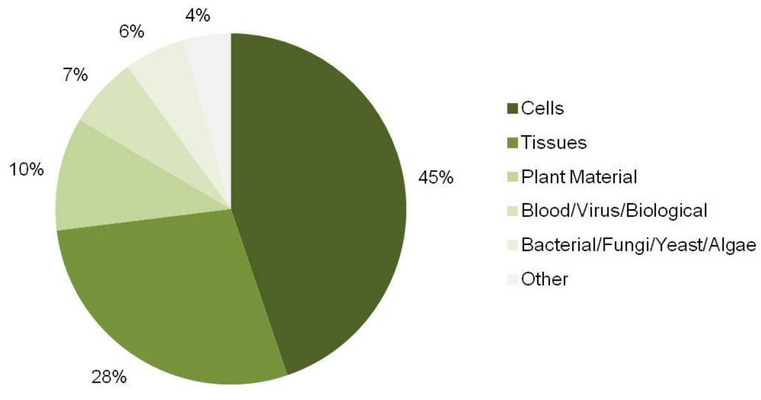
Sample sources

Cells from culture, solid tissue, plasma, serum, whole blood, and *in vitro* processed RNA *(e.g., transcription products, DNase-treated or labeled RNA)* or samples stored and preserved in *TRI-Reagent®* or similar.

Examples
**compatibility is not limited to samples listed; data based on large-scale beta test of Direct-zol™ RNA MiniPrep*

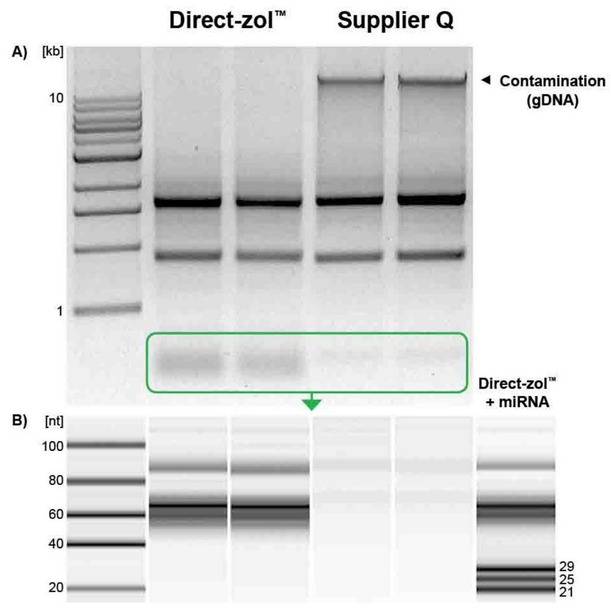
Tissue*	Brain (cerebrum, cerebellum)	Spleen	Oral cavity
	Spinal Cord	Retinal	Pedal ganglia
	Ovary	Lung	Paraffin sections of brain tissue
	Thyroid	Duodenum, Colon, Intestine	
	Joint	Pancreas	***plant tissue:***
	Liver	Tumor	Root
	Cortical	Lung	Leaves
	Mammary	Bursa of fabricius	Stem
	Uterine	Pituitary	Meristem
	Muscle (heart, skeletal, foot, buccal)	Adipose/Subcutaneous fat	Woody tissue
		Embryos	Rosettes
	Kidney (renal proximal tubule)	Placenta/umbilical cord	Callus
Organism*	Human	*Drosophila Melanogaster*	Arabidopsis thaliana
	Fish	*Aplysia (sea slug)*	Nicotiana benthaniana
	Mouse/Rat	Coral	Geranium maderense
	Chicken	Filarial Parasitic Nematode	Trifolium pretense
	Bovine	Clam	Lollium pyrenne
	Hydra		Austrodenthonia
	Mollusks		Medicago truncatula
Reagents	*TRI-Reagent®*, TRIzol®, RNAzol®, QIAzol®, TriPure, TriSure, RNA-Bee or similar acid-guanidinium-phenol based reagents		

Samples Typically used with Direct-zol™



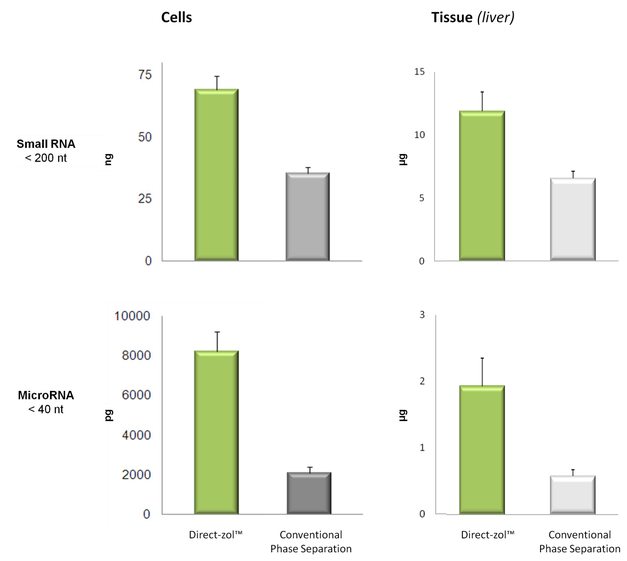
RESULTS

Broad Range RNA Recovery



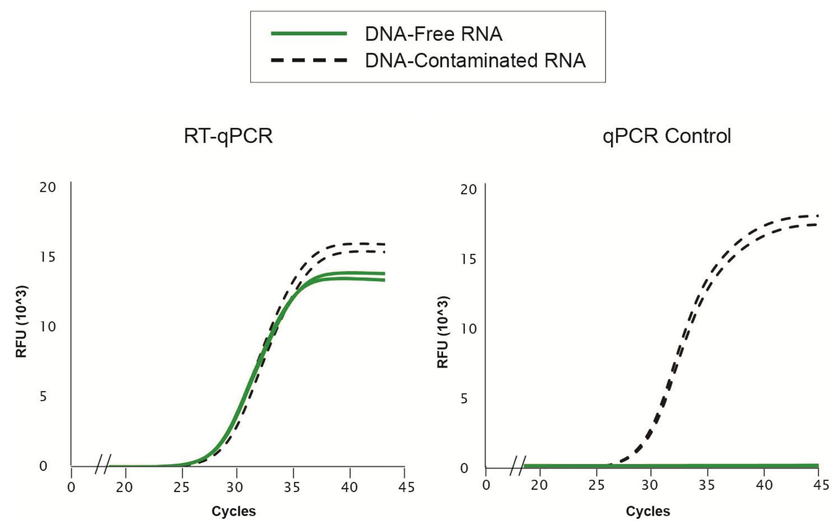
High quality broad range RNA purified with the Direct-zol™ RNA MiniPrep. (A) DNA-free RNA purified from human epithelial cells using the Direct-zol™ RNA MiniPrep compared to a preparation from Supplier Q (1% agarose/TAE). (B) Small RNAs are effectively recovered with the Direct-zol™ procedure while absent in Supplier Q preparations (Agilent Bioanalyzer 2100, Small RNA Chip data shown).

Efficient Recovery of Small RNAs



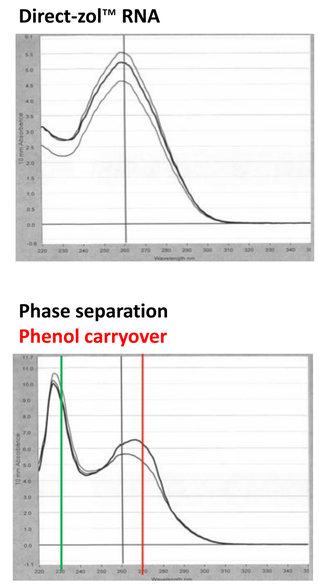
Robust small RNA recovery with Direct-zol™ RNA *(green)* compared to conventional phase separation *(gray)*. RNA was isolated from 10^4 human epithelial cells and from 10 mg mouse liver in TRI-Reagent®. Analysis was performed using an Agilent Bioanalyzer 2100 (Small RNA Chip data shown).

DNA-free RNA



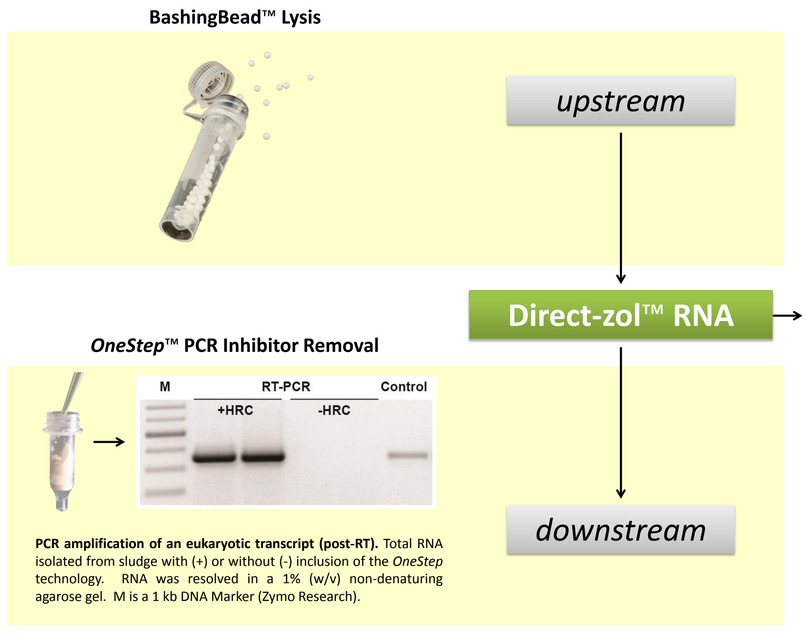
RT-qPCR amplification of a beta-actin transcript. DNA-free RNA was isolated from human epithelial cells (10^5 cells/prep) with the Direct-zol™ RNA MiniPrep that includes DNase I for in-column treatment (green). Non-DNase treated samples (black) are provided for comparison.

No Phenol Carryover

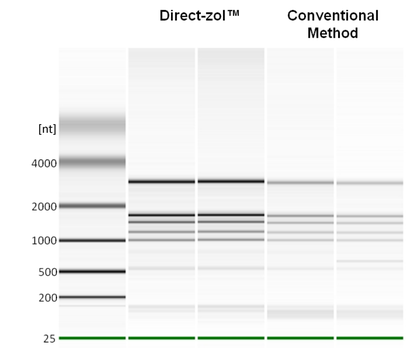


No phenol carryover with Direct-zol™ RNA. RNA isolated by conventional phase separation method shows phenol contamination as indicated by the absorption peak at 230nm (green) and peak shift 260->270nm (red).

Plant RNA



PCR amplification of an eukaryotic transcript (post-RT). Total RNA isolated from sludge with (+) or without (-) inclusion of the *OneStep* technology. RNA was resolved in a 1% (w/v) non-denaturing agarose gel. M is a 1 kb DNA Marker (Zymo Research).



Plant RNA is efficiently recovered using the Direct-zol™ RNA MiniPrep. RNA was isolated from leaf (9.7 mg; *Nicotiana sp.*) homogenized with BashingBeads in *TRI-Reagent®*. Analysis was performed with an Agilent Bioanalyzer 2100 (Nano Chip data shown). Isolation using conventional phase-separation is provided for comparison.

CONCLUSION

RT-PCR, hybridization techniques and next generation sequencing methods demand high quality RNA. The Direct-zol™ RNA MiniPrep is a next generation sample prep method that delivers high quality, broad range, DNA-free RNA from TRI-Reagent®, TRIzol® *etc.* Direct-zol™ bypasses phase separation with unique spin column and wash technologies making its application appealing for automated and high throughput workflows.

REFERENCES

1. Chomczynski, P. & Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162, 156-159 (1987)
2. Chomczynski, P. & Sacchi, N. The single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction: twenty-something years on. Nature Protocols 1(2), 581-585 (2006)
3. Ma, W. et al, Effect of long-term storage in TRIzol on microarray-based gene expression profiling. Cancer Epidemiol Biomarkers Prev. 19(10), 2445-2452 (2010)