Stabilization of biomolecules in various sample materials

Stefanie Schröer,[1] Christiane Bäumer,[1] Katharina Helmerichs,[2] and Vera Holländer[1]
[1] QIAGEN GmbH, R&D Department, Hilden, Germany
[2] QIAGEN Hamburg GmbH, R&D Department, Hamburg, Germany

Introduction

When collecting biological samples for gene expression analysis, it is critical to stabilize the samples immediately. Without stabilization, the profile of biomolecules such as DNA, RNA, and protein can change rapidly due to specific and nonspecific degradation, induction, and covalent modification. Sample stabilization is therefore a key step in the experimental workflow for gene expression analysis (Figure 1).

Sample stabilization is typically carried out by snap-freezing biological samples in liquid nitrogen. However, as handling liquid nitrogen and frozen samples is both hazardous and inconvenient, alternative methods for sample stabilization have been developed to overcome these disadvantages. These are reagents which are optimized for stabilizing a specific type of biomolecule in a particular sample type at room temperature (Table 1). We present a novel reagent, Allprotect Tissue Reagent, which stabilizes DNA, RNA, and protein in animal and human tissues without the need for freezing.

Figure 1. Experimental workflow for gene expression analysis.

Table 1. Methods for room-temperature sample stabilization

Method	Sample
Stabilization of DNA	
PAXgene™ Blood DNA Tubes	Blood
QIAcard™ FTA Spots	Various
Stabilization of RNA	
PAXgene Blood RNA Tubes	Blood
PAXgene Bone Barrow RNA Tubes	Bone marrow
RNA*later*® RNA Stabilization Reagent	Tissues
RNAprotect® Bacteria Reagent	Bacteria
RNAprotect Cell Reagent*	Cells
RNAprotect Saliva Reagent*	Saliva
Stabilization of protein	
No established standard yet	
Stabilization of DNA, RNA, and protein	
Allprotect Tissue Reagent	Tissues

* Reagent stabilizes both RNA and DNA.

Stabilization of RNA

Immediate stabilization of animal and human tissues in Allprotect Tissue Reagent ensures that RNA remains intact, even when the tissues are stored at room temperature for several days (Figure 3). Levels of mRNA transcripts and miRNAs are also preserved, allowing accurate quantification by real-time RT-PCR analysis (Figures 2 and 4).

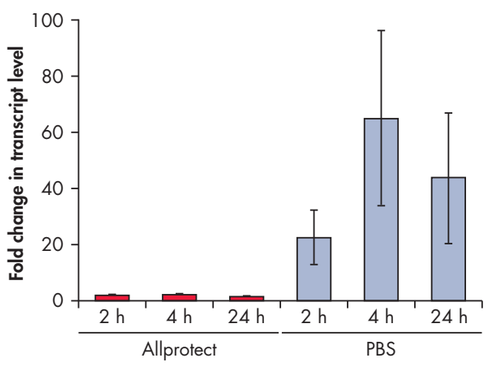

Figure 2. Prevention of gene induction after sample collection. Rat lung was stored at 25°C for 2, 4, or 24 hours in either Allprotect Tissue Reagent or PBS. RNA was then purified using the RNeasy® Mini Kit. Levels of c-fos transcript were quantified using the QuantiTect® SYBR® Green RT-PCR Kit and normalized to those for GAPDH transcript. The graph shows c-fos transcript levels relative to those in rat lung stored in liquid nitrogen.

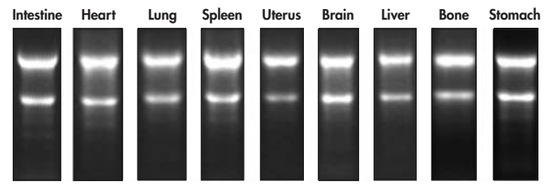

Figure 3. Effective stabilization of RNA in a variety of tissues. Various rat tissues were stored in Allprotect Tissue Reagent at 25°C for 3 days. Total RNA was purified from 10 mg tissue using the following kits: RNeasy Fibrous Tissue Mini Kit (heart); RNeasy Lipid Tissue Mini Kit (brain and bone); RNeasy Mini Kit (all other tissues). Purified RNA was analyzed on a 1% formaldehyde agarose gel.

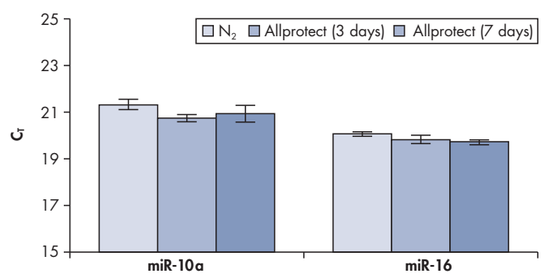

Figure 4. Effective stabilization of miRNA. Rat kidney was stored at 25°C for 3 or 7 days in Allprotect Tissue Reagent or in liquid nitrogen. RNA, including miRNA, was purified using the miRNeasy Mini Kit. Levels of miR-10a and miR-16 were quantified by real-time RT-PCR using the miScript System.

Stabilization of protein

Storage of animal and human tissues in Allprotect Tissue Reagent preserves protein integrity, allowing analysis of intact protein in applications such as western blotting and SELDI-TOF mass spectrometry (Figures 6 and 7). The reagent also preserves protein activity, enabling reliable enzyme assays (Figure 5).

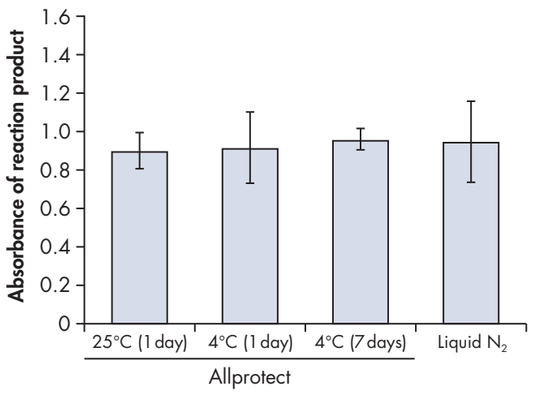

Figure 5. Preservation of protein activity. Rat liver was stored in Allprotect Tissue Reagent before total protein was purified using the Qproteome™ Mammalian Protein Prep Kit. Acidic phosphatase activity was similar to that of protein from control liver stored in liquid nitrogen. Each bar shows the mean from 2 liver pieces assayed in duplicate.

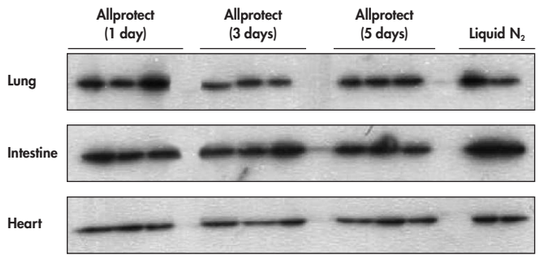

Figure 6. Intact protein for western blotting. Various rat tissues were stored in Allprotect Tissue Reagent at 25°C for up to 5 days; for comparison, tissues were also frozen in liquid nitrogen. Total protein was prepared using the Qproteome Mammalian Protein Prep Kit. Western blot analysis was carried out using an antibody against ERK2.

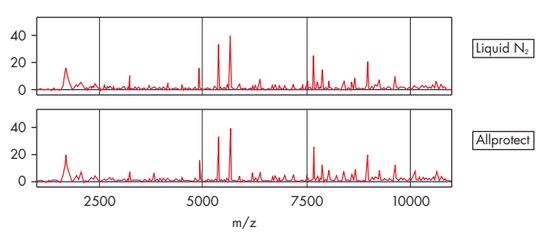

Figure 7. Intact protein for mass spectrometry. Human intestinal mucosa samples were stored for 1 day in Allprotect Tissue Reagent (2–8°C) or in liquid nitrogen, and then analyzed by SELDI-TOF MS. The example analysis shown demonstrates that the Allprotect sample gave similar peak patterns for <10 kDa protein as the liquid nitrogen sample. (Data kindly provided by Indivumed GmbH, Hamburg, Germany.)

Stabilization of modified DNA and protein

Storage of animal and human tissues in Allprotect Tissue Reagent also protects modifications of biomolecules, allowing reliable analysis of DNA methylation (Figure 8) and protein phosphorylation (Figure 9).

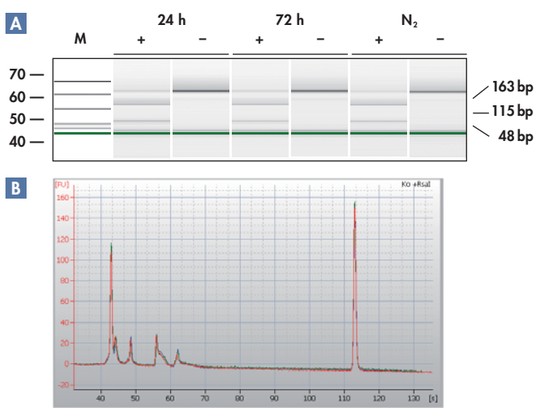

Figure 8. Preservation of the DNA methylation pattern. Rat liver was stored for 1 or 3 days in Allprotect Tissue Reagent (25°C) or in liquid nitrogen. Genomic DNA was purified using the EZ1® DNA Tissue Kit, and then bisulfite converted using the EpiTect™ Bisulfite Kit. A 163-bp amplicon of the line1 transposon was amplified, and then either restriction digested with RsaI into 115-bp and 48-bp fragments (+) or left untreated (–). Only methylated sequences are cut by RsaI. **A** Size analysis on the Agilent® 2100 bioanalyzer. **B** The electropherograms of all "+" samples show close overlap, demonstrating similar methylation status in all samples. **M**: markers.

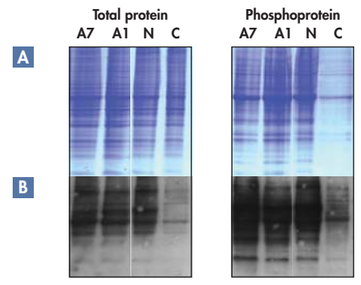

Figure 9. Preservation of the protein phosphorylation pattern. Rat brain was stored in Allprotect Tissue Reagent for 1 day (**A1**) or 7 days (**A7**); in liquid nitrogen for 1 day (**N**); or in 0.8% NaCl for 1 day as a negative control (**C**). The PhosphoProtein Purification Kit was used to isolate total protein and to enrich for phosphoproteins from 2.5 mg total protein. **A** SDS-PAGE and **B** western blotting with an antiserine and antithreonine antibody (42H2 from Cell Signaling) were carried out using 20 µg total protein and 1/3 of the pooled phosphoprotein fractions.

Long-term stabilization of RNA and protein

Biological samples can be safely stored in Allprotect Tissue Reagent at 2–8°C for up to 1 year. Under these conditions, no degradation of RNA and protein is observed (Table 2 and Figure 10).

Table 2. Stabilization of RNA for 12 months without freezing

Storage (months)	RIN value		
	Liver	Intestine	Brain
1	9.4	9.3	8.5
2	9.2	9.3	8.6
4	9.4	9.3	8.4
6	9.2	9.5	8.6
9	9.4	9.7	8.2
12	9.4	9.3	8.4

Various rat tissues were stored in Allprotect Tissue Reagent at 2–8°C for up to 12 months. Total RNA was purified using the RNeasy Mini Kit (liver and intestine) or RNeasy Lipid Tissue Mini Kit (brain), and then analyzed on the Agilent 2100 bioanalyzer.

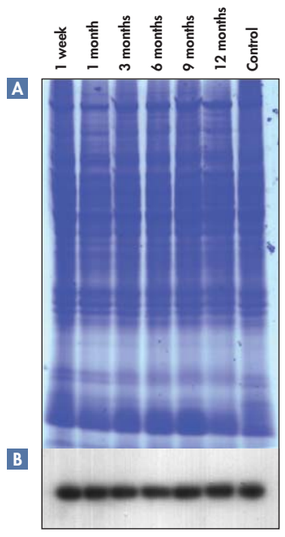

Figure 10. Stabilization of protein for 12 months without freezing. Rat liver was stored in Allprotect Tissue Reagent at 2–8°C for up to 12 months. Total protein was prepared using the Qproteome Mammalian Protein Prep Kit. **A** SDS-PAGE and **B** western blotting with anti-ERK2 was carried out. As a control, a liver sample was snap-frozen in liquid nitrogen and stored at –80°C.

Summary and conclusions

- All biological samples must be stabilized upon collection to prevent changes in the gene expression profile
- Reagents for stabilizing RNA or DNA are available for various sample types
- Allprotect Tissue Reagent is the first reagent to simultaneously stabilize DNA, RNA, and protein in tissue samples

Table 3. Features of Allprotect Tissue Reagent

Feature
Simultaneous stabilization of DNA, RNA, and protein
Immediate stabilization of harvested tissues
Storage of samples at 37°C for 1 day; at 25°C for up to 7 days; at 2–8°C for up to 12 months
Archiving of samples at –20°C or –80°C
Compatibility with a wide range of analyte purification procedures and downstream assays
Nontoxicity

PAXgene Blood RNA Tubes are intended for in-vitro diagnostic use. The RNeasy Mini Kit, RNAprotect Saliva Reagent, and EZ1 DNA Tissue Kit are intended for general laboratory use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease. All other products are intended for research use. No claim or representation is intended to provide information for the diagnosis, prevention, or treatment of a disease.

"RNA*later*®" is a trademark of AMBION, Inc., Austin, Texas and is covered by various U.S. and foreign patents.

1053052 05/2008