Welcome to the QIAGEN Seminar

Standardization from Sample Collection to Nucleic Acid Isolation: A Prerequisite for Reliable Molecular Analysis

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Diagnostic Sample Preparation & Stabilization
QIAGEN GmbH
The Molecular Diagnostic Process

Pre-Analytical Steps

Collection → Transport → RNA/DNA Purification

Analytical Steps

Assay → Detection Data

Detection

Data

Assay

RNA/DNA Purification

Transport

Collection

RNA/DNA

Purification

Assay

Detection

Data

Innovation Working for You
What happens to RNA in Biological Samples ex vivo?

- **Induction**
- **in vivo transcript level at \( t_0 \)**
- **Cell death / enzymatic degradation**

Sample collection

Time

mRNA copy number
Transcription Profile Changes in Unpreserved Whole Blood Stored at Room Temperature

Data provided courtesy of Source Precision Medicine, Boulder, CO
Integrated Solutions for a Wide Range of Starting Materials

- Blood
  - PAXgene™ Blood RNA System
  - PAXgene™ Blood DNA System

- Tissue
  - RNeasy® Protect System for Tissue
  - RNAlater™ TissueProtect Tubes

- Bacteria
  - RNeasy® Protect Bacteria Kits
PAXgene™ Blood RNA System

For Research Use Only

Innovation Working for You
Gene Expression Profile Preserved with PAXgene

Source Precision Profile™ for Inflammation Subset
Detection of Circulating Tumor Cells in Whole Blood by RT PCR

Positive detections out of 12 independant experiments

Data provided courtesy of N. S. Prang, Micromet, Munich
RNAlater™ TissueProtect Tubes

- Standardization of tissue collection
- Time-zero preservation of cellular RNA and DNA at point of collection
- Room temperature stability of intracellular RNA and DNA for 7 days
- Sample stabilization during transport and storage
- System integrated RNA + DNA isolation

For Research Use Only
Stability of RNA in Rat Kidney Samples

<table>
<thead>
<tr>
<th>Unstabilized</th>
<th>Stabilized in RNA Later</th>
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<tbody>
<tr>
<td>0  5  10  15  30  60</td>
<td>0  5  10  15  30  60</td>
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<td>minutes</td>
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Agarose gel and Northern Blotting analysis
Convenient Sample Handling and Processing without Liquid Nitrogen

- Sample processing at RT
- No liquid nitrogen or dry ice
- Storage, transport at RT or 4°C
- Archiving at -20°C or -80°C

RNeasy Protect Stabilization
Crucial Factors for Sample Collection, Transport and Storage

- No RNA degradation
- No down-regulation of genes
- No gene induction
- Integrated standardized systems

Accurate gene expression results
Crucial Factors for RNA Purification

- Sample type and size
- Throughput: manual / automation
- RNA elution volume
- RNA yield
- RNA quality
  - Integrity
  - Purity
- RNA assay performance
Small Samples: Special Solutions needed

RNeasy Micro Kit

- Special Silica Membrane Spin Column
  - low 2 µl dead volume

- Low elution volume: 10 µl

- RNA isolation from small samples
  - ≤ 10 µg up to 5 mg tissue
  - 1 single cell up to 5 x 10^5 cells

- LMD, FNA, Biopsies
  - Functional Genomics
  - Target Identification
  - Oncology Research
  - Molecular Diagnostics
Integrity of RNA: Rabbit Liver Fine Needle Aspirate

- RNA*later* TissueProtect Tubes
- Total RNA by RNeasy Micro
- Agilent 2100 BioAnalyser

<table>
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<th>Fragment</th>
<th>Name</th>
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<th>End_Time(secs)</th>
<th>Area</th>
<th>%_of_total_Area</th>
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<td>rRNA Ratio [28S / 18S]</td>
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<td>2,32</td>
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Linearity of RNA Yield: Low Cell Numbers

RNeasy Micro Kit: 1 – 4,100 HeLa Cells

A  RT-PCR on LightCycler

B  Linear RNA yield
Fatty Tissues: Special Solutions Needed

RNeasy Lipid Tissue Kit

- Fat compounds interfere with many RNA isolation methods
  - Risk of low yield
  - Risk of inhibitors in RNA

- Lysis by Phenol/GITC based QIAzol Reagent

- RNA Purification by Silica Membran Technology
  - Eliminates risk of phenol carry over into RNA eluates
Integrity and Purity of Rat Brain RNA

Formaldehyde agarose gel, equal volume per lane

Agilent 2100 BioAnalyser

UV spectrophotometry

4.7 11.2 14.0 µg RNA

28S 18S

Small RNAs

LMW RNAs

M RNeasy Phenol

— 28 S

— 18 S

Contaminants

Phenol
The RNeasy Technology - Advantages

- standardized, reliable
- fast, easy to handle and reproducible
- low and high-throughput, fully automated
- free of contaminants (reliable quantitation, no inhibition of enzymes)
- high-quality RNA

Accurate real-time gene expression results
Choose the RNA kit for your application!

<table>
<thead>
<tr>
<th>Sample source</th>
<th>RNeasy Micro Kit</th>
<th>RNeasy Kits</th>
<th>RNeasy Protect Kits</th>
<th>RNeasy Fibrous Tissue Kits</th>
<th>QIAamp RNA Blood Kits</th>
<th>PAXgene Blood RNA System</th>
<th>RNeasy Protect Bacteria Kits</th>
<th>RNeasy Plant Kits</th>
<th>RNeasy/MinElute Cleanup Kit</th>
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<td>Small cell and tissue samples</td>
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<td>Standard tissues</td>
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<td>Standard tissues, plus stabilization</td>
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<td>Fiber-rich tissues (e.g., heart, muscle, skin)</td>
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<td>Whole blood, plus stabilization</td>
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<td>Bacteria, plus stabilization</td>
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<td>RNA cleanup and concentration</td>
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