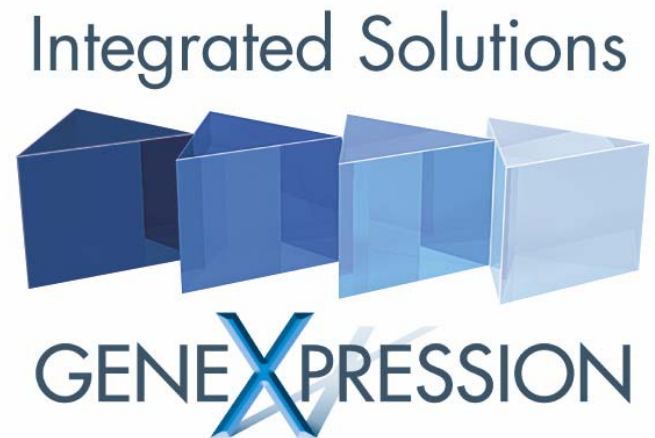


Welcome to the QIAGEN Seminar

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

Ralf Wyrich, PhD
Senior Scientist R&D
Diagnostic Sample Preparation & Stabilization
QIAGEN GmbH



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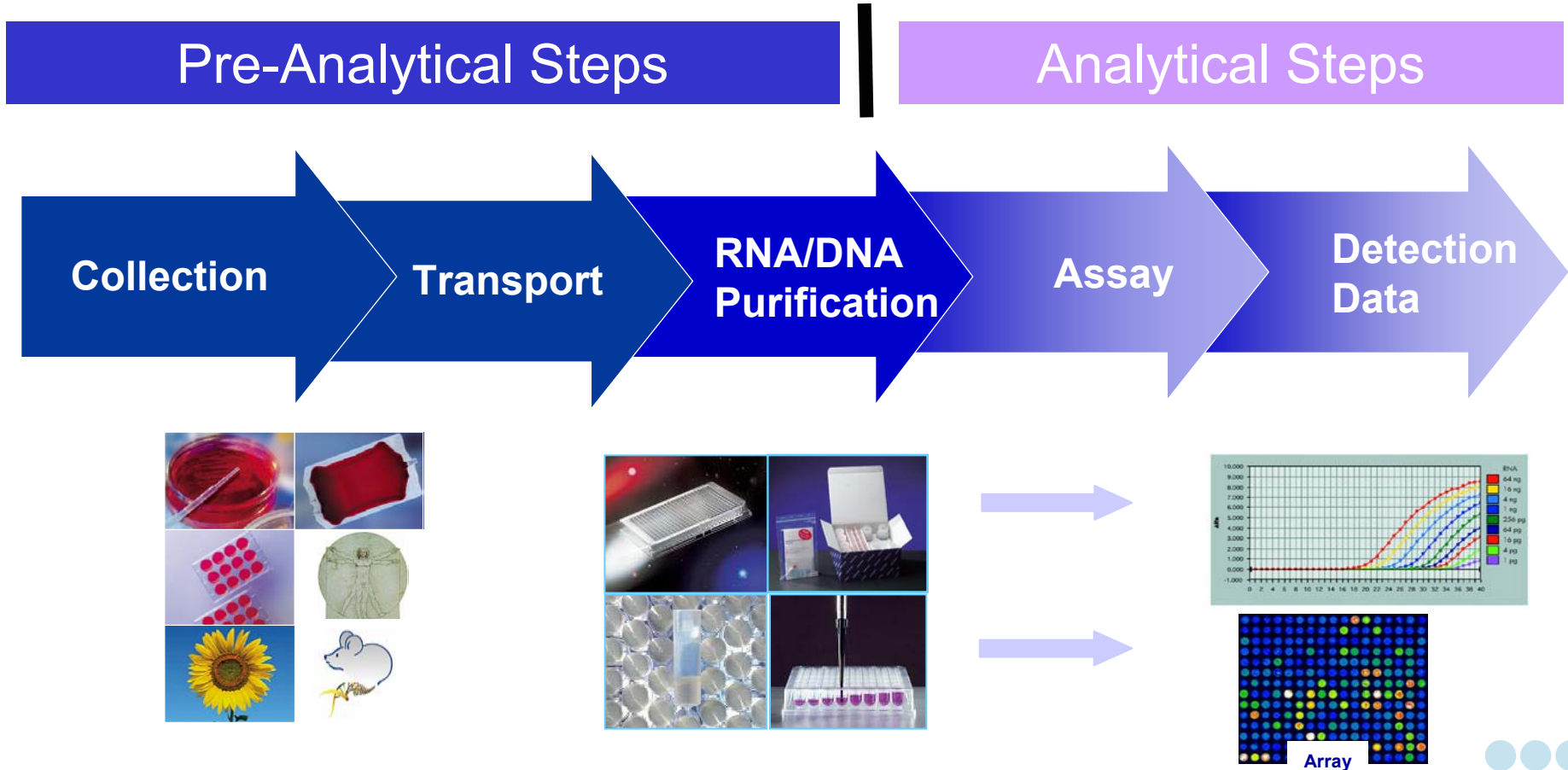


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The Molecular Diagnostic Process



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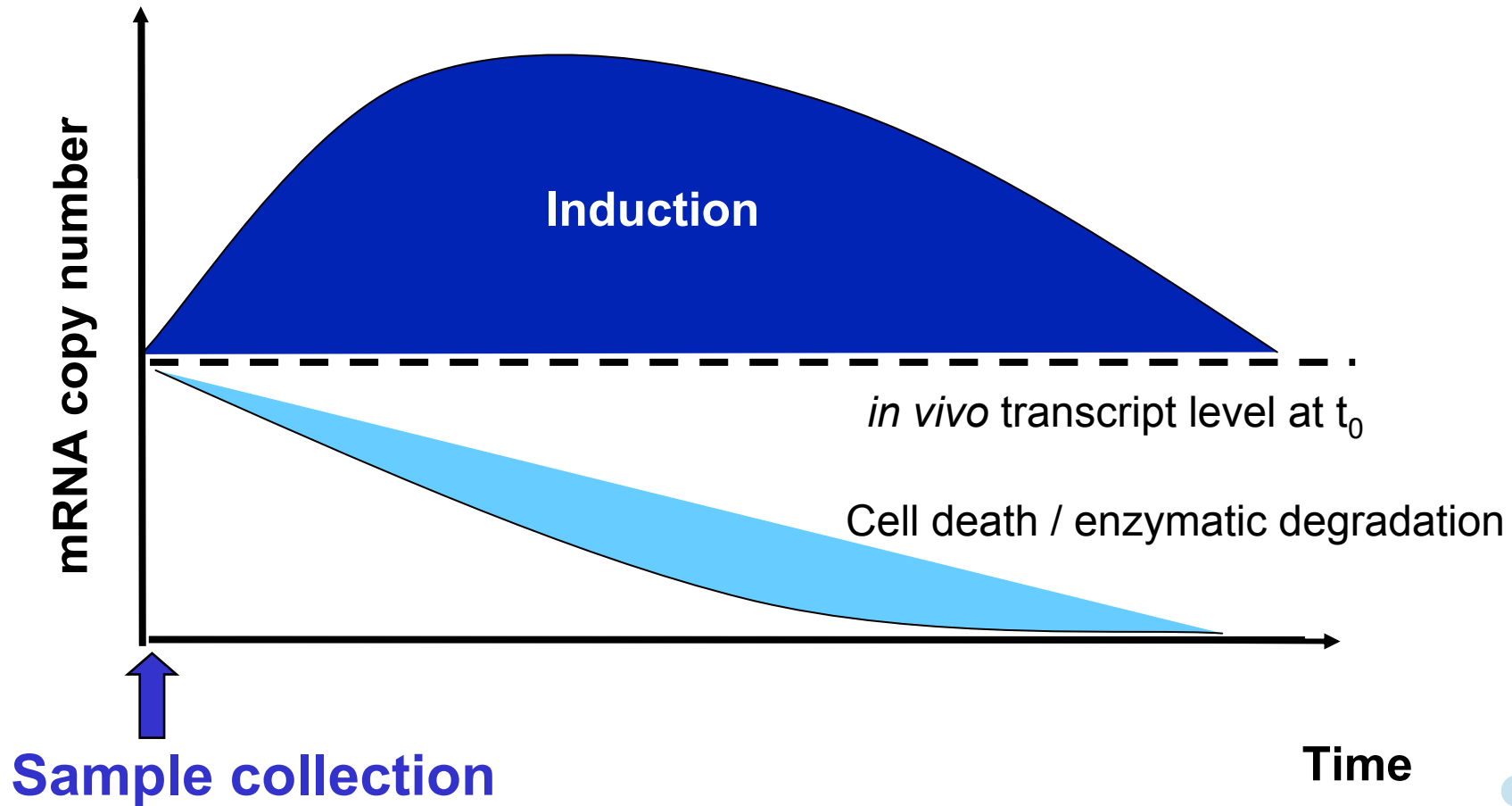


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What happens to RNA in Biological Samples *ex vivo*?



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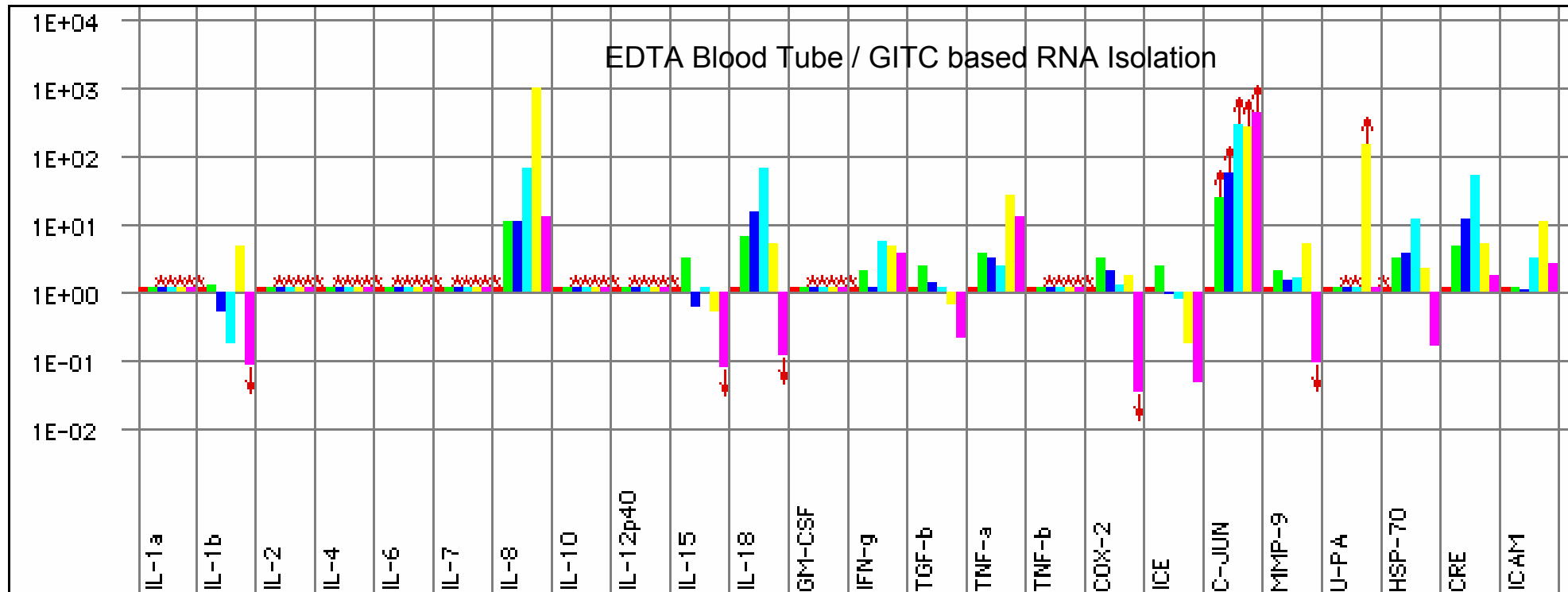


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Transcription Profile Changes in Unpreserved Whole Blood Stored at Room Temperature



Norm.
Copy No.

■ t₀
■ 4hr
 ■ 8hr
 ■ 24hr
 ■ 3 days
 ■ 5 days

⬆ Value displayed is a minimum

⬆ Value displayed is a maximum

⬆ Not enough signal to calculate ²Ct reliably

Data provided courtesy of Source Precision Medicine, Boulder, CO

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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

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PAXgene™ Blood RNA System



For Research Use Only

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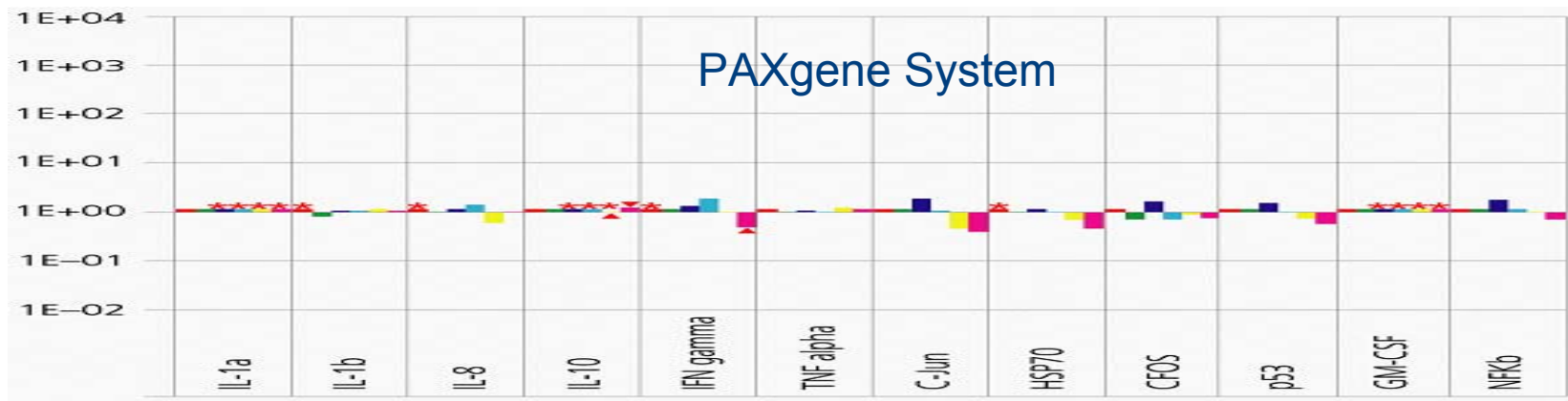
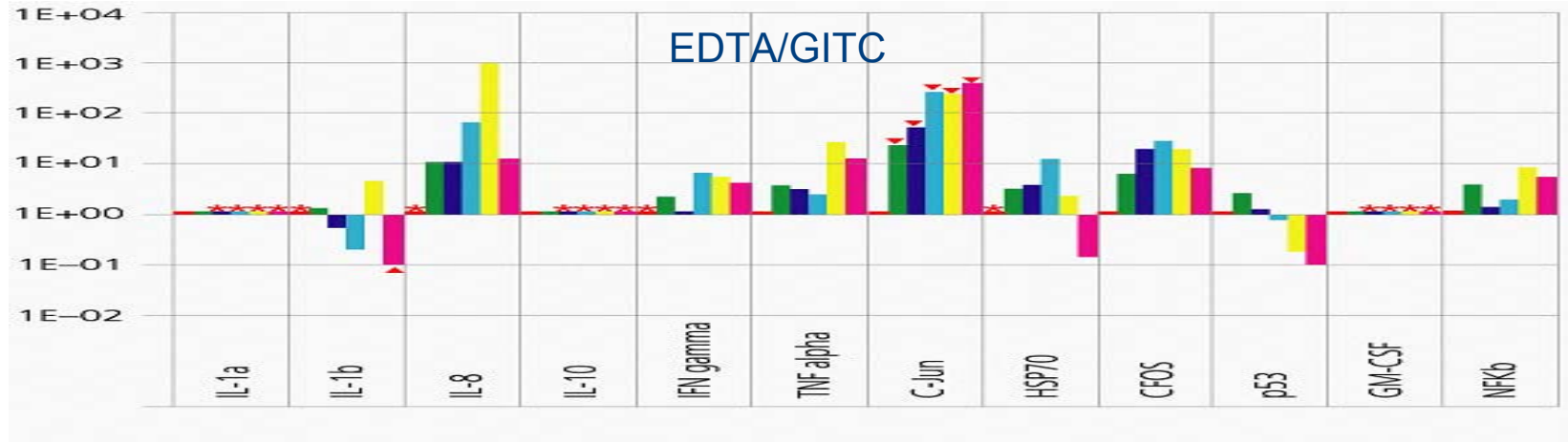


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Gene Expression Profile Preserved with PAXgene



Source Precision Profile™ for Inflammation Subset

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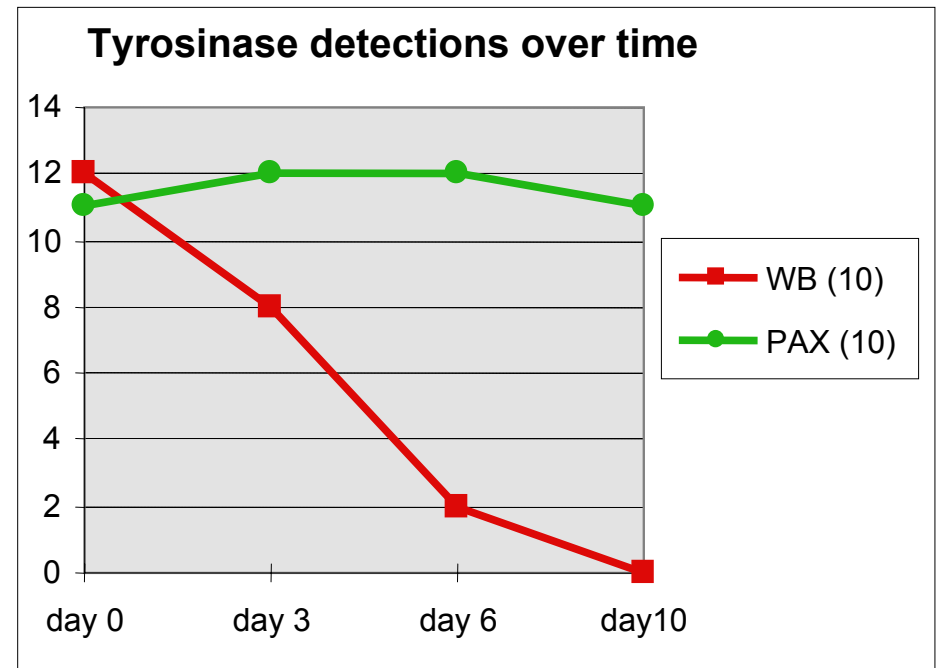
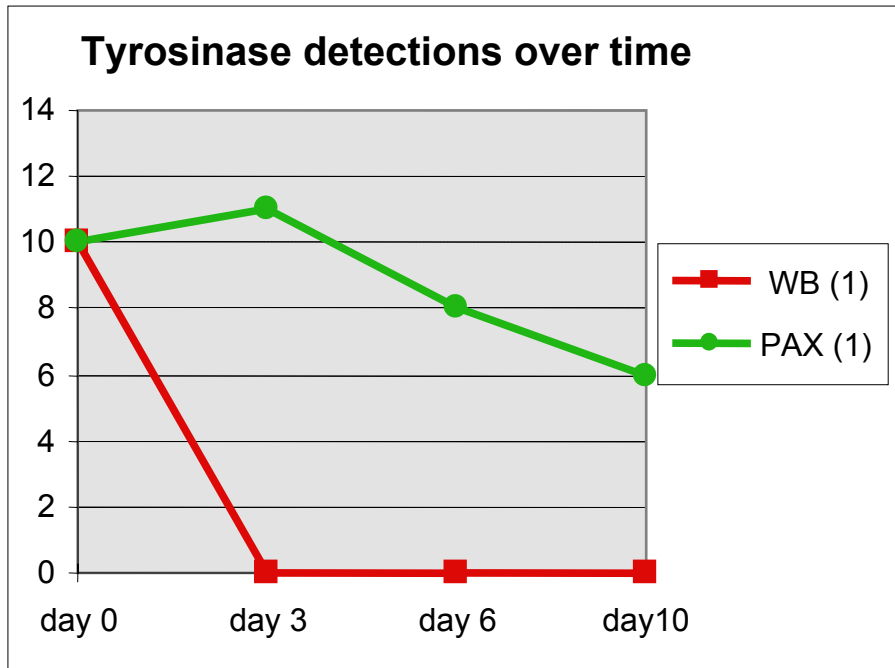
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Detection of Circulating Tumor Cells in Whole Blood by RT PCR



Positive detections out of 12 independant experiments



Data provided courtesy of N. S. Prang, Micromet, Munich

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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

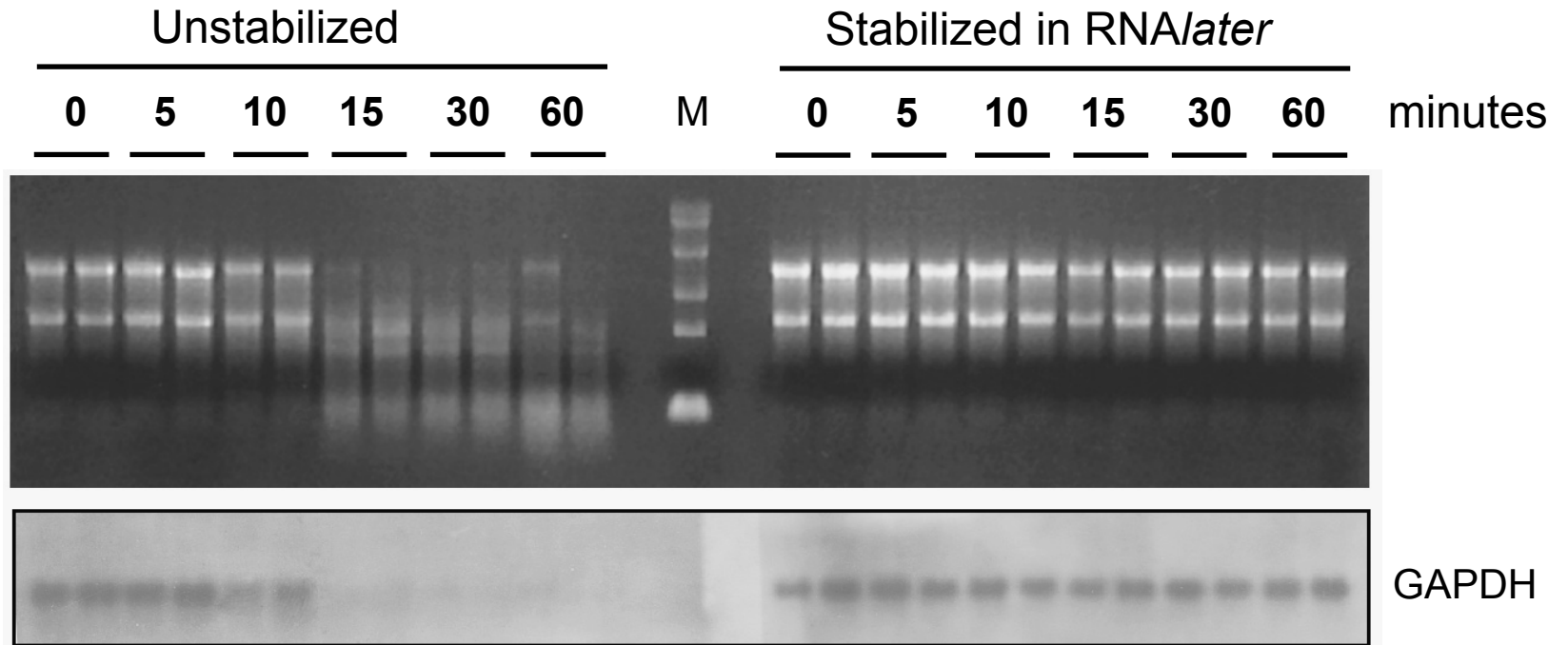
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Stability of RNA in Rat Kidney Samples



Agarose gel and Northern Blotting analysis

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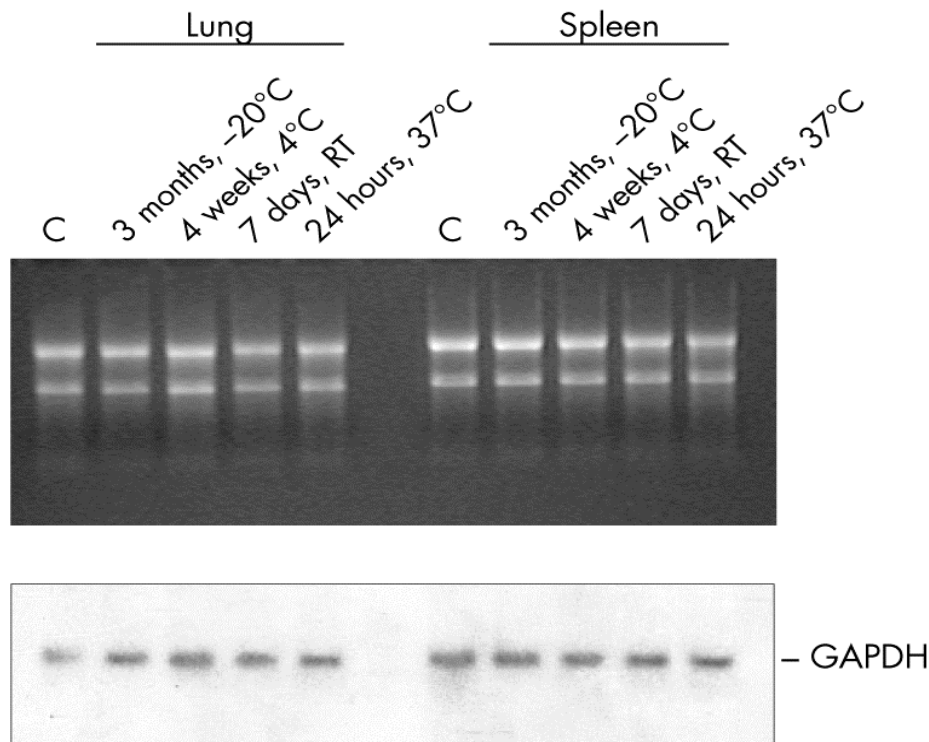


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Convenient Sample Handling and Processing without Liquid Nitrogen



- 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

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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

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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



- LMD, FNA, Biopsies
 - Functional Genomics
 - Target Identification
 - Oncology Research
 - Molecular Diagnostics

RNeasy Micro Kit

- Special Silica Membrane Spin Column
 - low 2 μ l dead volume
- Low elution volume: 10 μ l
- RNA isolation from small samples
 - \leq 10 μ g up to 5 mg tissue
 - 1 single cell up to 5×10^5 cells

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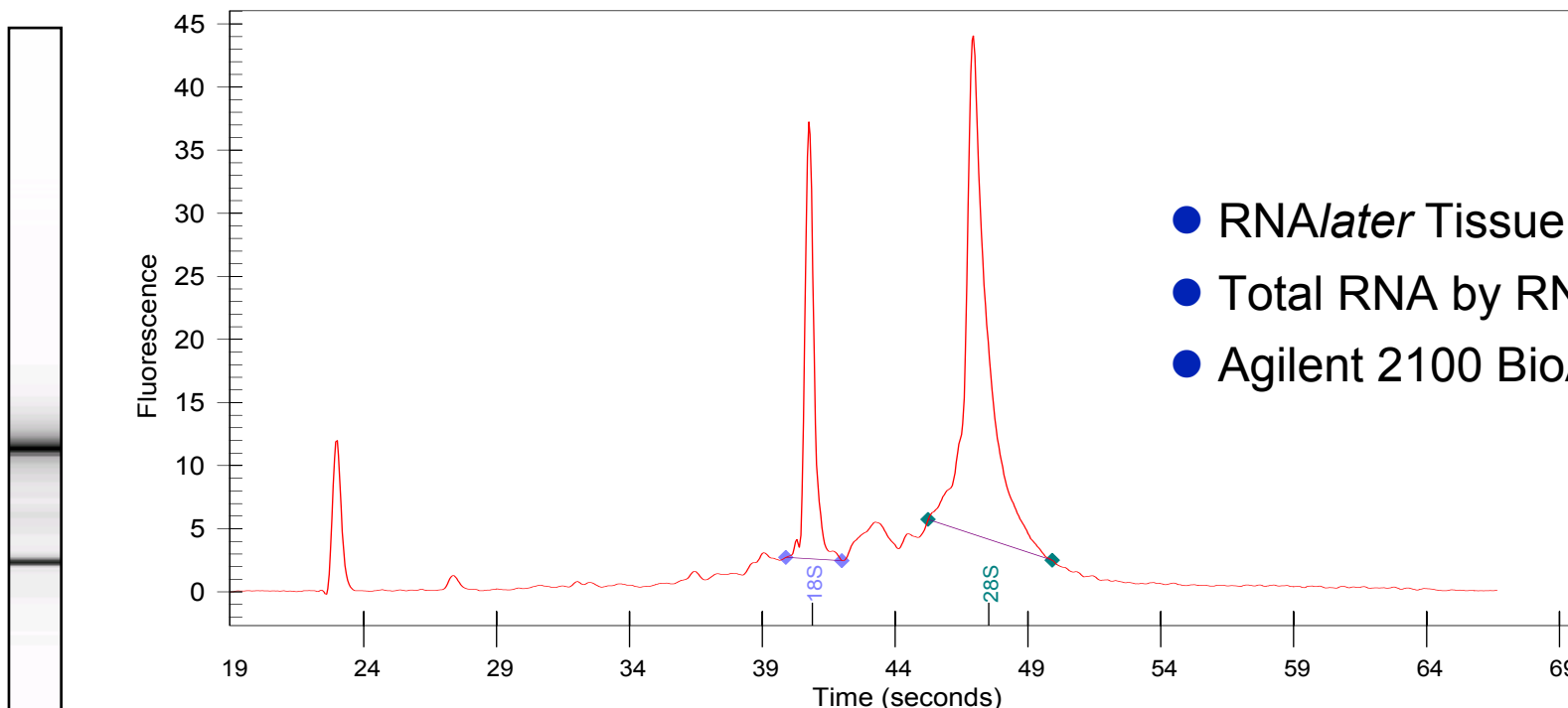


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Integrity of RNA: Rabbit Liver Fine Needle Aspirate



Fragment	Name	Start_Time(secs)	End_Time(secs)	Area	%_of_total_Area	
1	18S	39,88	42,00	42,00	34,44	14,05
2	28S	45,23	49,92	49,92	79,82	32,56
RNA Area	245,13					
RNA Concentration(ng/ul)	90,20					
rRNA Ratio [28S / 18S]	2,32					

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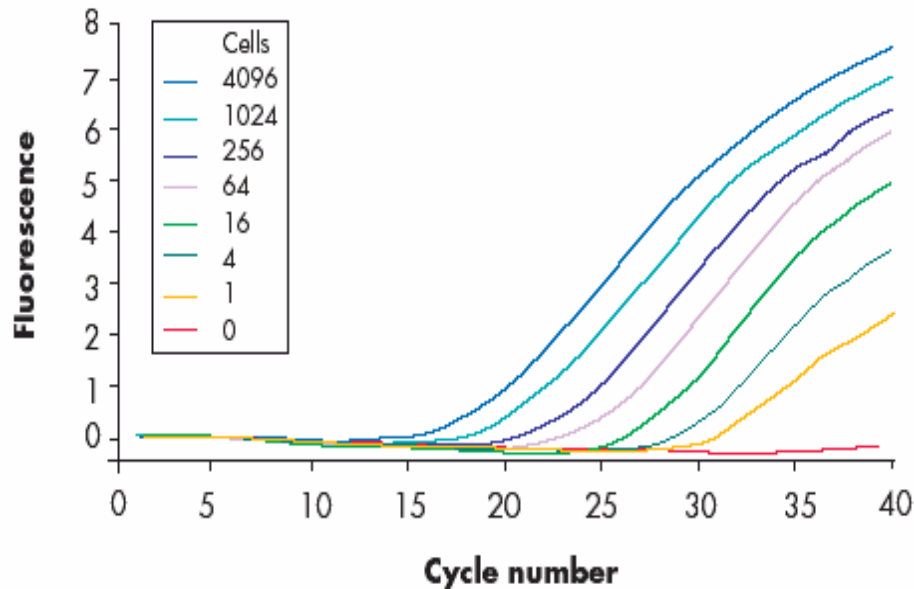
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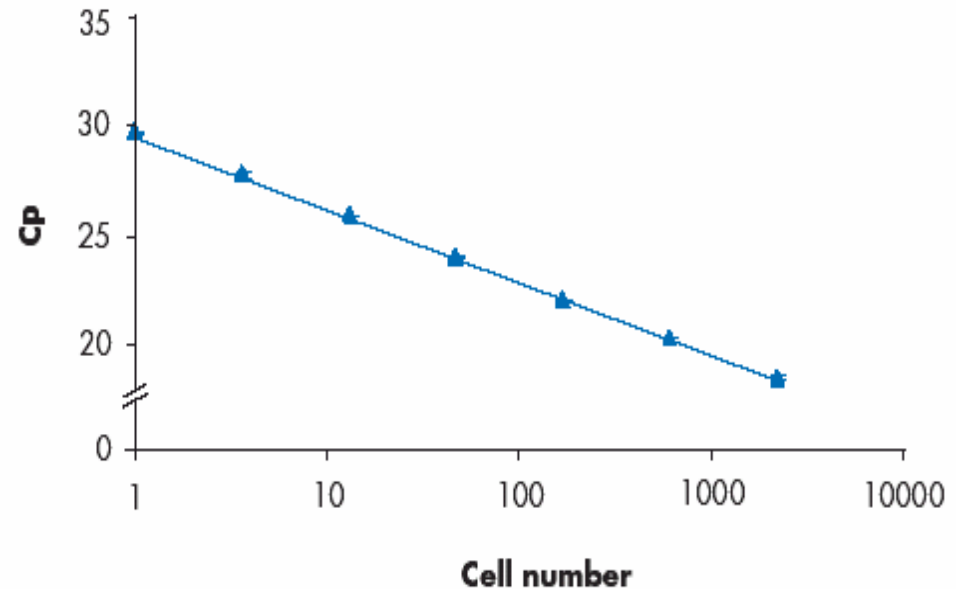
Linearity of RNA Yield: Low Cell Numbers

RNeasy Micro Kit: 1 – 4.100 HeLa Cells

A RT-PCR on LightCycler



B Linear RNA yield



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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

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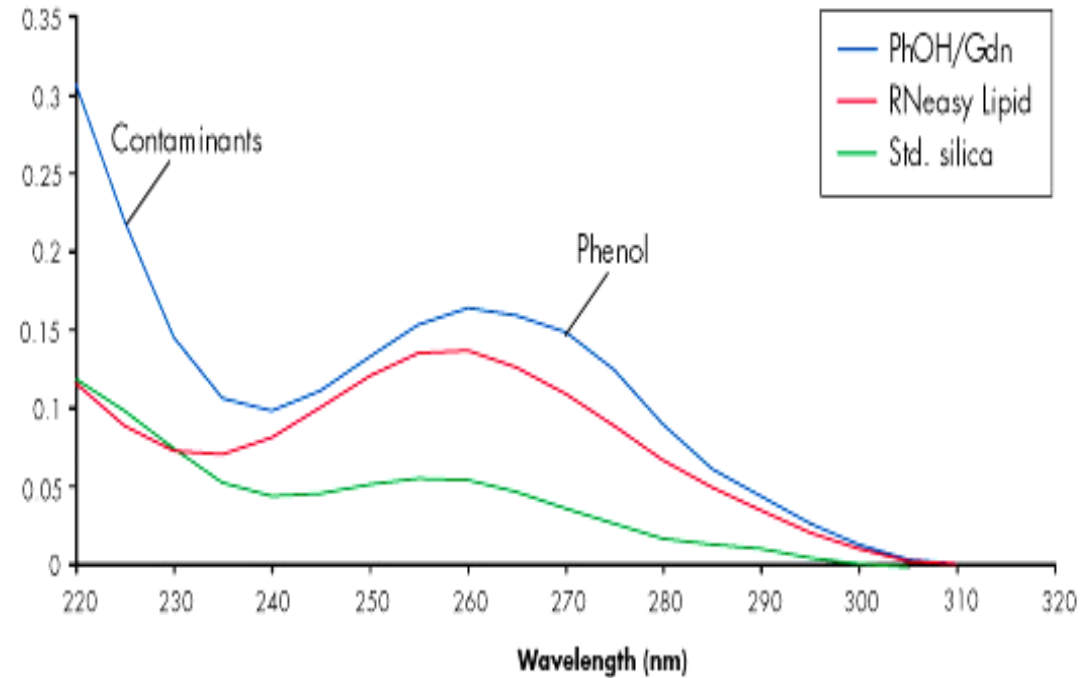
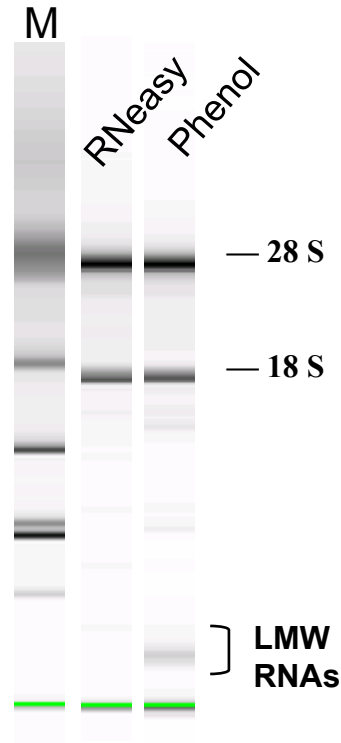
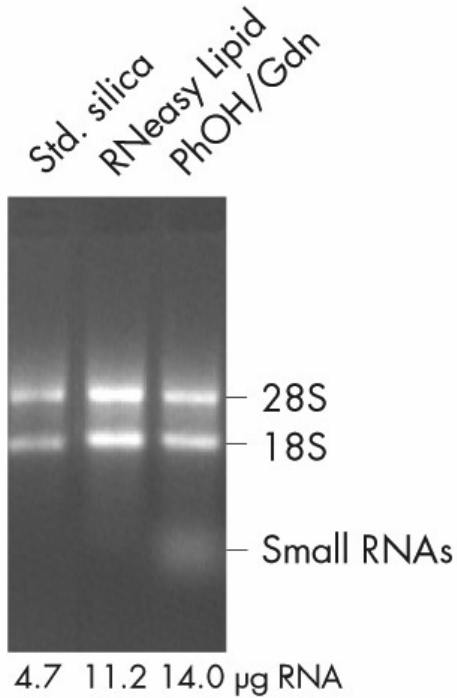


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Integrity and Purity of Rat Brain RNA



Formaldehyde agarose gel,
equal volume per lane

Agilent 2100
BioAnalyser

UV spectrophotometry

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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

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Choose the RNA kit for your application!

Sample source	RNeasy [®] Micro Kit*	RNeasy Kits	RNeasy Protect Kits	RNeasy Fibrous Tissue Kits*	RNeasy Lipid Tissue Kits*	QIAamp [®] RNA Blood Kits*	PAXgene [™] Blood RNA Kits	RNeasy [™] Blood RNA System	RNeasy Protect Bacteria Kits	RNeasy Plant Kits	RNeasy MinElute [™] Cleanup Kit
Small cell and tissue samples (e.g., FACS [®] , FNA, LMD)	●										
Standard tissues (e.g., kidney, liver, spleen)		●	●	●	●						
Standard tissues, plus stabilization			●								
Fiber-rich tissues (e.g., heart, muscle, skin)		●	●	●	●						
Fatty tissues (e.g., adipose tissue, brain)		●	●	●	●						
Cells		●									
Whole blood					●						
Whole blood, plus stabilization							●				
Bacteria, plus stabilization								●			
Yeast		●									
Plants								●			
RNA cleanup and concentration	●	●									●

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