

# Total RNA from cells and tissue

RNA sample preparation



## Legal disclaimer



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## Intact, high-quality total RNA from cells and tissue – kit overview



Sample		Kit	Features	Processing	Sample amount	Format	Elution volume
			Single-spin, silica techr	nology			
Mini spin kits for cells and tissue		RNeasy Plus (50, 250)	Fast gDNA removal, gDNA removal columns included	Manual/ centrifugation or QIAcube	<10 <sup>7</sup> cells or 30 mg tissue	Mini column	30–50 μL
		RNeasy (50, 250)	The gold standard for RNA isolation from cells and tissue	Manual/ centrifugation or QIAcube	10 to 1 x 10 <sup>7</sup> animal or human cells, 0.5–30 mg animal or human tissues	Mini column	30–100 μL
		QlAwave Plus RNA Mini (50, 250)	FCO-triandiar varsion of the PNIAgev Plus Mini Kit		Mini column	30–50 μL	
		QIAwave RNA Mini (50, 250)	Eco-friendlier version of the RNeasy Mini Kit	Manual/ centrifugation or QIAcube	10 to 1 x 10 <sup>7</sup> animal or human cells, 0.5–30 mg animal or human tissues	Mini column	30–100 μL
		RNeasy Mini QIAcube (240)	Specifically designed for use with QIAcube Connect – spin columns and tubes already loaded onto the rotor	QIAcube Connect	<1 x 10 <sup>7</sup> cells, < 30 mg tissue, 20–250 mg animal or human tissue	Mini column	30–100 μL
	Include stabilization solution (RNAprotect)	RNeasy Protect (50, 250)	50 mL RNAprotect Tissue Reagent included	Manual/ centrifugation or QIAcube	0.5–30 mg animal or human tissues	Mini column	30–100 μL
		RNeasy Protect Cell Mini (50)	50 mL RNAprotect Cell Reagent included	Manual/ centrifugation or QIAcube	Max. 10 <sup>7</sup> cells	Mini column	30–100 μL
	For difficult-to- lyse tissues	RNeasy Fibrous Tissue (50)	Optimized protocols for fibrous tissues, with Proteinase K to remove structural proteins	Manual/ centrifugation or QIAcube	0.5–30 mg of fiber-rich tissue samples	Mini column	30–100 μL
		RNeasy Plus Universal Mini (50)	QIAzol lysis and RNeasy purification, includes gDNA Eliminator Solution	Manual/ centrifugation	25–50 mg tissue samples, including difficult-to-lyse and demanding tissues	Mini column	30 µL
Micro kits for sma and elution volum		RNeasy Plus Micro (50)	Optimized for small samples and elution volumes, fast gDNA removal, gDNA removal columns included	Manual/ centrifugation or QIAcube	<5 x 10 <sup>5</sup> cells, <5 mg tissue	Micro column	14 µL
8		RNeasy Micro (50)	Optimized for small samples and elution volumes	Manual/ centrifugation or QIAcube	<5 x 10 <sup>5</sup> cells, <5 mg tissue	Micro column	10–14 μL
		RNeasy UCP Micro (50)	Ultra-clean production (UCP) of spin columns and buffers for RNA-seq of low abundance targets	Manual/ centrifugation or QIAcube	<5 mg tissue or 5 x 10 <sup>5</sup> cells	Micro column	10–20 μL

## Intact, high-quality total RNA from cells and tissue – kit overview



Sample	Kit	Features	Processing	Sample amount	Format	Elution volume
Midi and maxi kits for larger samples	RNeasy Midi (50)	Midi format RNeasy kit	Manual/ centrifugation	5 x 10 <sup>6</sup> to 1 x 10 <sup>8</sup> animal or human cells, 20–250 mg tissue	Midi columns	300–500 μL
	RNeasy Maxi (20)	Maxi format RNeasy kit	Manual/ centrifugation	Up to 5 x $10^8$ animal or human cells, 150 mg to 1 g tissue	Maxi columns	800–2400 μL
		96-well,	silica technology			
	RNeasy Plus 96 (12 x 96)	96-well version of RNeasy Plus kit	Manual or 3rd party, Centrifugation, QIAvac/ Centrifugation	Fast gDNA removal, gDNA removal plates included	96-well plate	45–140 μL
	RNeasy 96 (4 x 96 / 12 x 96)	96-well version of RNeasy Mini kit	Manual or 3 <sup>rd</sup> party, Centrifugation, QIAvac/ Centrifugation	10–5 x 10 <sup>5</sup> cells	96-well plate	45–140 μL
	RNeasy 96 Universal Tissue (4 x 96)	QIAzol lysis and RNeasy 96 purification	Manual or 3 <sup>rd</sup> party, Centrifugation, QIAvac/ Centrifugation	25–50 mg Tissue samples including difficult-to- lyse and demanding tissues	96-well plate	45–70 μL
		Kits for a	utomation platforms			
Silica plate/	RNeasy Mini QIAcube (240)	Specifically designed for use with QIAcube Connect - spin columns and tubes already loaded onto the rotor	QIAcube Connect	<1 x 10 <sup>7</sup> cells, < 30 mg tissue	Mini columns	30–100 μL
column based	QIAcube HT RNA (5x 96), QIAzol Lysis Reagent (200 mL)	Automated purification of total RNA or total RNA, including miRNA, from animal and human cells and tissue samples on the QIAcube HT system	QIAcube HT	Up to 5 x $10^5$ cells, homogenized in 140 $\mu L$ Buffer RLT	96-well plate	110 µL
	EZ2 RNA/miRNA Tissue/Cells (48)	Automated purification of RNA and miRNA from human and animal cells and tissues on the EZ2	EZ2 Connect	Up to 30 mg (frozen) or 15 mg (stabilized) tissue or 10 mg frozen spleen or lung tissue, or up to 5 x 10 <sup>6</sup> cells	Cartridge/ mag beads	50 or 100 μL
Magnetic bead based	EZ1&2 RNA Tissue Mini (48)	For automated purification of high-quality total RNA	<u>EZ2/ EZ1</u>	5–10 μg tissue	Cartridge/ mag beads	50–200 μL
	QIAsymphony RNA (192)	Automated purification of total RNA, or total RNA including miRNA and other small RNAs, from 1–96 samples on the QIAsymphony SP	<u>QlAsymphony</u>	For 2 x 96 samples: $\leq 3 \times 10^6$ cultured cells, $\leq$ 20 mg easy-to-lyse tissue, $\leq$ 20 mg fibrous tissue, For 2 x 48 samples: $3 \times 10^6$ – $1 \times 10^7$ cultured cells $\leq$ 50 mg easy-to-lyse tissue	Cartridge/ mag beads	50 or 100 or 200 μL

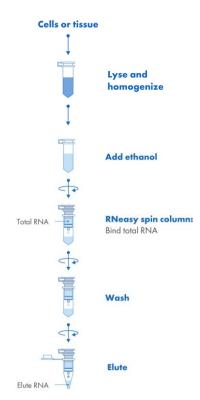
## RNeasy – the most trusted kit for total RNA extraction from cells and tissue



## Ready-to-use RNA for high performance in any downstream application

- Consistent RNA yields from small to large amounts of starting material
- No phenol/chloroform extraction, CsCl gradients, or LiCl/ethanol precipitation
- Simple "lyse-bind-washelute" protocol
- High-throughput processing in 96-well format and automatable protocols

	RNeasy Micro	RNeasy Mini	QIAwave RNA Mini	RNeasy Mini QIAcube	RNeasy 96	RNeasy Midi	RNeasy Maxi
Format	Micro column	Mini column	Mini column	Mini column	96-well plate	Midi columns	Maxi columns
Target				RNA <200 nt	:		
Sample	<5 x 10 <sup>5</sup> cells, <5 mg tissue	10 to 1 x 10 <sup>7</sup> cells, 0.5–30 mg tissues	10 to 1 x 10 <sup>7</sup> cells, 0.5–30 mg tissues	<1 x 10 <sup>7</sup> cells, <30 mg tissue	10–5 x 10 <sup>5</sup> cells	5 x 10 <sup>6</sup> to 1 x 10 <sup>8</sup> animal or human cells	Up to 5 x 10 <sup>8</sup> cells, 150 mg to 1 g tissue
Elution volume	10–14 μL	30–100 μL	30–100 μL	30–100 μL	45–140 μL	300–500 μL	800–2400 μL
Processing	Manual	(centrifugation), a	utomated	Automated	Manual (centrifugation or centrifugation /vacuum)	5 x 10 <sup>6</sup> to 1 x 10 <sup>8</sup> cells, 20–250 mg tissue	Up to 5 x 10 <sup>8</sup> cells, 150 mg to 1 g tissue
Automation	QIAcub	e Connect		QIAcube Connect	3rd party	-	-
gDNA removal	Optional o			al on-column DN	lase digest		





Did you know that there are more than 300,000 peer-reviewed citations for RNeasy? Most of them for RNeasy Mini?

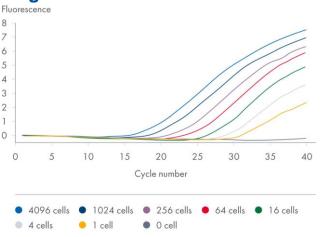
Total RNA from cells and tissue

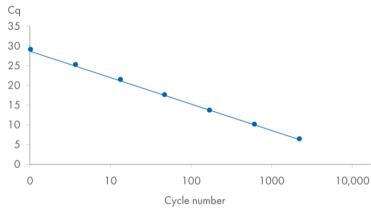
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## RNeasy – the most trusted kit for total RNA extraction from cells and tissue



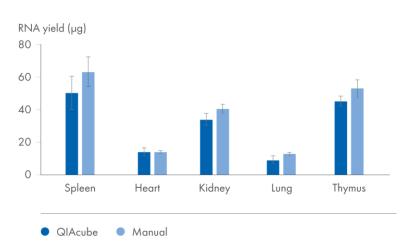
## Linear and very sensitive down to a single cell





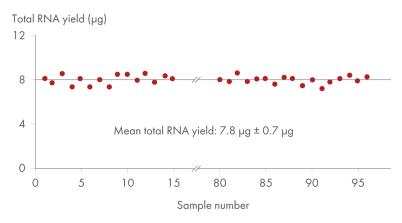
 Total RNA was isolated from the indicated number of HeLa cells using the RNeasy Micro Kit

## High RNA yield from various tissues – manual and automated



- Rat tissue (15 mg) was stabilized in RNAprotect Tissue Stabilization Solution and disrupted using the TissueLyser III
- RNA was purified using the RNeasy Mini kit, either manually or on the QIAcube Connect

## Reproducible purification of high-quality RNA



- HeLa cells (5 x 10<sup>5</sup>) from a homogeneous cell culture were pelleted in a 96-well cell-culture plate and the total RNA purified using the RNeasy 96 Kit
- Each square represents the yield from a preparation originating from 1 well of the 96-well cell culture plate

## RNeasy Plus – the fastest way to remove gDNA



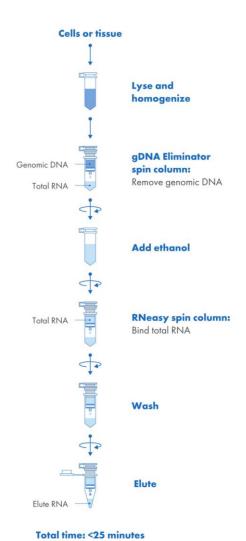
# Efficient gDNA removal with unique gDNA Eliminator columns or plates (no need for DNase)

- High-quality total RNA in minutes using fast and simple extraction protocols
- Phenol-free RNA isolation
- High-throughput processing in 96-well format
- Ideal for sensitive applications such as real-time RT-PCR and RNA-seq

	RNeasy Plus Micro	RNeasy Plus Mini	QIAwave RNA Plus Mini	RNeasy Plus 96
Format	Micro column	Mini column	Mini column	96-well plate
Target	RNA <200 nt	RNA <200 nt	RNA <200 nt	RNA <200 nt
Sample material	<5 x 10 <sup>5</sup> cells or 5 mg tissue	<10 <sup>7</sup> cells or 30 mg tissue	<10 <sup>7</sup> cells or 30 mg tissue	10 to 5 x 10 <sup>5</sup> cells
Elution volume	10–14 μL	30–100 μL	30–100 μL	30–50 μL
Processing	Manu	al (centrifugation), autor	nated	Manual (centrifugation or centrifugation/vacuum)
Automation		QIAcube Connect		-
gDNA removal	gDNA Eliminator Columns		gDNA Eliminator Plate	
Processing time		25 min		-



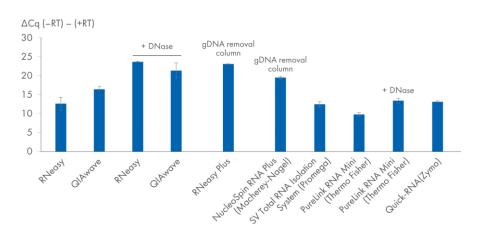
Did you know? QIAGEN invented the spin-column technology more than 30 years ago and revolutionized how nucleic acids are purified from any biological sample.



## RNeasy Plus – the fastest way to remove gDNA

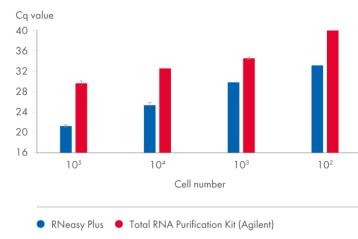


#### **Effective removal of genomic DNA**



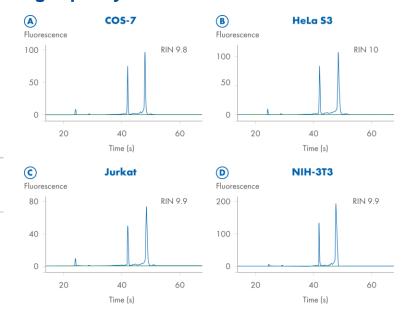
- Total RNA was purified in duplicate from mouse liver (10 mg per sample) using QIAGEN Kits or kits from other suppliers
- Real-time PCR assays were performed to determine the amount of DNA contamination in the purified RNA

#### High, reproducible RNA yields



- Total RNA was purified in duplicate from different amounts of Jurkat cells using the RNeasy Plus Mini Kit or a similar kit from another supplier
- Real-time RT-PCR assays for β-actin were performed (40 cycles)
- The lower Cq values with the RNeasy Kit demonstrate greater RNA yields. With the Total RNA Purification Kit, no transcript was detectable in RNA purified from 10<sup>2</sup> cells

#### **High-quality RNA**



- Total RNA was purified from the indicated cell lines using the RNeasy Plus 96 Kit and analyzed on the Agilent 2100 Bioanalyzer
- The RIN values were close to 10, indicating the high integrity of the purified RNA

Data obtained through experiments conducted by QIAGEN R&D, Hilden , Germany.

## Save time and enjoy greater convenience with RNeasy Plus Kits



#### RNeasy vs. RNeasy Plus

	RNeasy		RNeasy Plus	
gDNA removal	Optional on-column or post- isolation DNase treatment	_	gDNA Eliminator columns	++++
Preparation time	Longer procedure when combined with on-column digest	_	Fast procedure due to gDNA eliminator column/plate (no need for DNase)	+ + +
Popularity	More than 300,000 citations, most popular RNA isolation kit on the market	++++	More than 27,400 citations	++
Formats	Highest flexibility in yield and throughput: Micro, Mini, Midi, Maxi scale and 96-well format	++++	Micro, Mini and 96-well	+
QIAwave	Yes, 50 and 250 preps		Yes, 50 and 250 preps	
Applications	Ideal for applications that do not require removal of residual gDNA		Ideal for DNA sensitive applications such as RNA-seq	

#### Save ~6 working days per year by switching to RNeasy Plus



#### Did you know?

Additional genomic DNA removal is unnecessary for many analytical methods. qPCR primers spanning exon-intron boundaries and cDNA synthesis kits with integrated genomic DNA removal, like QuantiNova Reverse Transcription Kit, do not require additional gDNA removal.

Make sure to select the RNA sample prep kit that best meets your needs.

#### Want less lab waste and more space?

Did you know that using QIAwave RNA and QIAwave RNA Plus—the eco-friendlier versions of RNeasy and RNeasy Plus—can save up to 62% plastic and 58% cardboard?

View website

## Avoiding RNA degradation – RNeasy Protect Kits



#### For stabilization and purification of total RNA from tissues, cells, human saliva and bacteria

- Specialized stabilization solution for cells and tissue combined with proven RNeasy RNA purification
- Immediate stabilization of RNA and RNA protection
- Reliable gene expression and geneprofiling data

#### **RNeasy Protect Kits – sample stabilization plus purification**

	Cultured cells	Tissue	Bacteria	Animal blood	Saliva
Kit name	RNeasy Protect Cell Mini Kit	RNeasy Protect Mini Kit	RNeasy Protect Bacteria Mini Kit	RNeasy Protect Animal Blood System	RNeasy Protect Saliva Mini
Sample type	Cultured cells	Animal or human tissue	Gram-positive and gram-negative bacteria	Animal blood	Saliva
Stabilization	RNAprotect Cell Reagent	RNAprotect Tissue Reagent	RNAprotect Bacteria reagent (2 x 100 mL)	Contains Animal Blood Tubes	RNAprotect Saliva Reagent
RNA extraction kit	RNeasy Mini	RNeasy Mini	RNeasy Mini	RNeasy Animal Blood	RNeasy Micro
Input	Max. 10 <sup>7</sup> cells	0.5-30 mg tissue	Up to 7.5 x 10 <sup>8</sup>	100-500 μL blood	200 μL saliva
	PNAprotect Call	RNAprotect Tissue	RNAprotect Bacteria	RNAprotect Animal Blood tubes also	
Special features	RNAprotect Cell Reagent also available separately	Reagent also available separately	Reagent also available separately	available separately, protocol for purification of small RNA available	-

## 

RNA was isolated from fresh rat kidney samples after 0, 5, 10, 15, 30, and 60 minutes, using either unstabilized samples or RNeasy Protect Kits. M: markers.

#### **Visit website**

#### Did you know?

RNA degradation is a significant risk that arises from endogenous RNases present in tissues and cells. To prevent RNA degradation, it is crucial to inactivate these RNases immediately upon harvesting tissues. **RNAprotect** reagents are designed to quickly permeate tissues or cells and stabilize and protect RNA. This means that you don't have to process your samples right away.

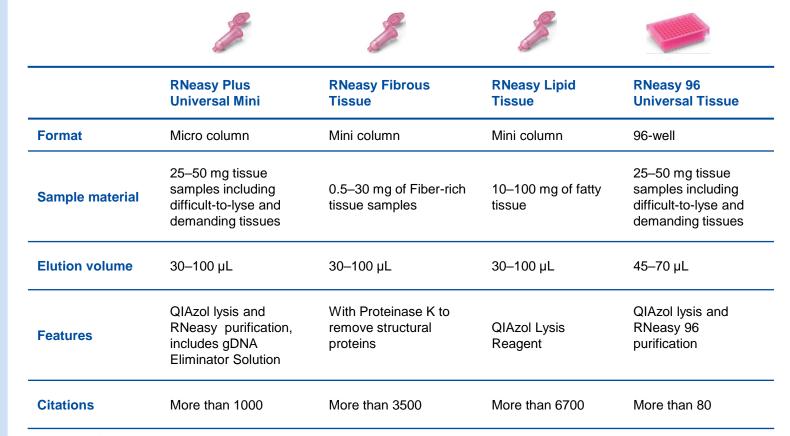
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### Kits for difficult-to-lyse tissues



# Dedicated RNeasy kits for special sample types and difficult to lyse tissues

- Developed for difficultto-lyse samples (e.g., fiber- or lipid-rich tissue)
- Deliver high-quality nucleic acids, ready for downstream use
- Optimized protocols enable purification of high-quality RNA from any type of tissue, even difficult-to-lyse tissues.





Check out our <u>RNA Benchguide</u> for recommendations on disruption and homogenization

## Tying to isolate RNA from difficult-to-lyse tissues?

Tissues high in fat (e.g., brain, breast, and adipose tissue), fiber (e.g., skin, heart, and skeletal muscle), or RNases (e.g., pancreas) can pose significant challenges for RNA isolation. QIAGEN offers a variety of optimized kits for such samples, helping you get highquality RNA—even from the most challenging samples.

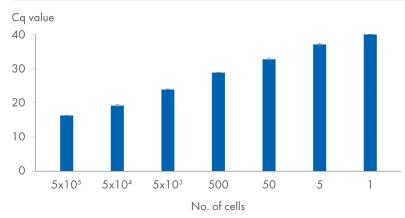
## Achieve high yields, even from small sample quantities



#### Focus Product: RNeasy UCP Micro

- Ultra-Clean Production (UCP) of spin columns and buffers
- RNA isolation without exogenous nucleic acids for RNA-Seq of low abundance targets
- Consistent RNA yields from very small amounts of starting material

	RNeasy UCP Micro	RNeasy Plus Micro	RNeasy Micro	
Sample For limited sample quantities like LCM issues, fine-needle aspirates,		es, fine-needle aspirates, and FACS-sorted	CS-sorted cells	
Sample amount	<5 mg tissue, <5 x 10 <sup>5</sup> cells	<5 mg tissue, <5 x 10 <sup>5</sup> cells	<5 mg tissue, <5 x 10 <sup>5</sup> cells	
Elution volume	10–20 μL	14 μL	10–14 μL	
gDNA removal	On column digest, RNase-free DNase, included in kit	gDNA eliminator column, non enzymatic	On column digest, RNase-free DNase, included in kit	
Features	Ultra-Clean Production (UCP) of spin columns and buffers	Fast gDNA removal	RNeasy optimized for small samples	
Applications	Low biomass samples, RNA-Seq of low abundance targets	Suited for downstream applications that are sensitive to low amounts of DNA contamination	Quantitative, real-time RT-PCR and other standard applications	



#### Linearity over a broad dilution range

Total RNA was purified from 10-fold serial dilutions of Jurkat cells using the RNeasy UCP Micro Kit. The  $\beta$ -actin transcript was detected even down to one cell by real-time RT-PCR, and reproducible CT values were observed at each dilution, indicating the absence of inhibition

#### Did you know?

Sorting small cell populations directly into the lysis buffer of the RNA isolation kit can enhance RNA integrity\*

\*Loontiens S, et al. Purification of high-quality RNA from a small number of fluorescence-activated cell-sorted zebrafish cells for RNA sequencing purposes. BMC Genomics. 2019;20(1):228.

## Automated RNA purification from cells and tissue



	·»))	·)))			
Instrument	QIAcube Connect	EZ2 Connect	QIAcube HT	QIAsymphony	
Throughput	Up to 12 samples	Up to 24 samples	Up to 96 samples	Up to 96 samples	
Kits	RNeasy Mini QIAcube (240)	EZ2 RNA/miRNA Tissue/Cells (48)	QIAcube HT RNA (5x 96)	QIAsymphony RNA	
	Most RNeasy kits*	EZ1&2 RNA Tissue Mini (48)**			
Sample amount	<1 x 10 <sup>7</sup> cells, <30 mg tissue	Up to 30 mg (frozen) or 15 mg (stabilized) tissue or 10 mg frozen spleen or lung tissue, or up to 5 x 10 <sup>6</sup>	Up to 5 x 10 <sup>5</sup> cells, homogenized in 140 μL Buffer RLT	For 2 x 96 samples: ≤3 x 10 <sup>6</sup> cultured cells, ≤20 mg easy-to-lyse tissue, ≤20 mg fibrous tissue,	
		cells		For 2 x 48 samples: 3 x 10 <sup>6</sup> –1 x 10 <sup>7</sup> cultured cells	
Format	Single-spin columns	Cartridge/magnetic beads	96-well plate	Cartridge/magnetic beads	
Elution volume	30–100 μL	50 or 100 μL	110 µL	50, 100 or 200 μL	

<sup>\*</sup> Most RNA Single-Spin kits can be processed on the QIAcube Connect. For available protocols please check here: QIAcube Connect standard protocols - QIAGEN

<sup>\*\*</sup> Will be replaced by EZ2 RNA/miRNA Tissue/Cells (48)

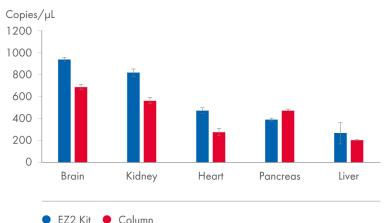
## High degree of automation with the EZ2 RNA/miRNA Tissue/Cells



# Purification of microRNA and total RNA from formalin-fixed, paraffin-embedded tissue sections

- Proven QIAGEN chemistry allows efficient recovery of RNA
- Efficient removal of gDNA guarantees unimpaired downstream analysis
- Prefilled and sealed cartridges ensure processing without risk for contamination
- Parallel processing of up to 24 samples minimizes intra-run variability between samples of one batch

#### dPCR – high RNA concentration

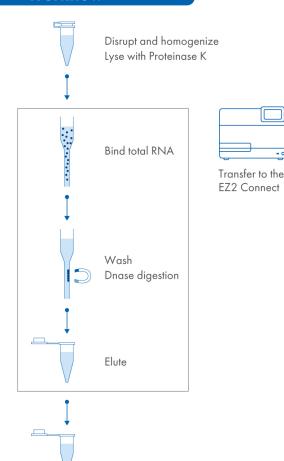


- Total RNA was purified from stabilized rat tissues using the EZ2 RNA/miRNA Tissue/Cell Kit and a manual columnbased system. Digital PCR was performed
  - High RNA concentration were consistently observed in samples purified with the EZ2 RNA/miRNA Tissue/Cell Kit

## Total RNA was purified from 10 mg stabilized rat tissues

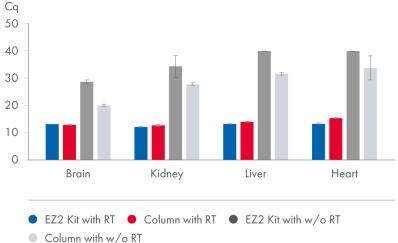
- 10 mg stabilized rat tissues using the EZ2 RNA/miRNA Tissue/Cell Kit and the miRNeasy Tissue/Cells Advanced Mini Kit
- Real-time RT-PCR for pgk1 was performed with or without reverse transcriptase. Both kits show comparable mRNA recovery and good gDNA removal

### EZ2 RNA/miRNA Tissue/Cells Workflow



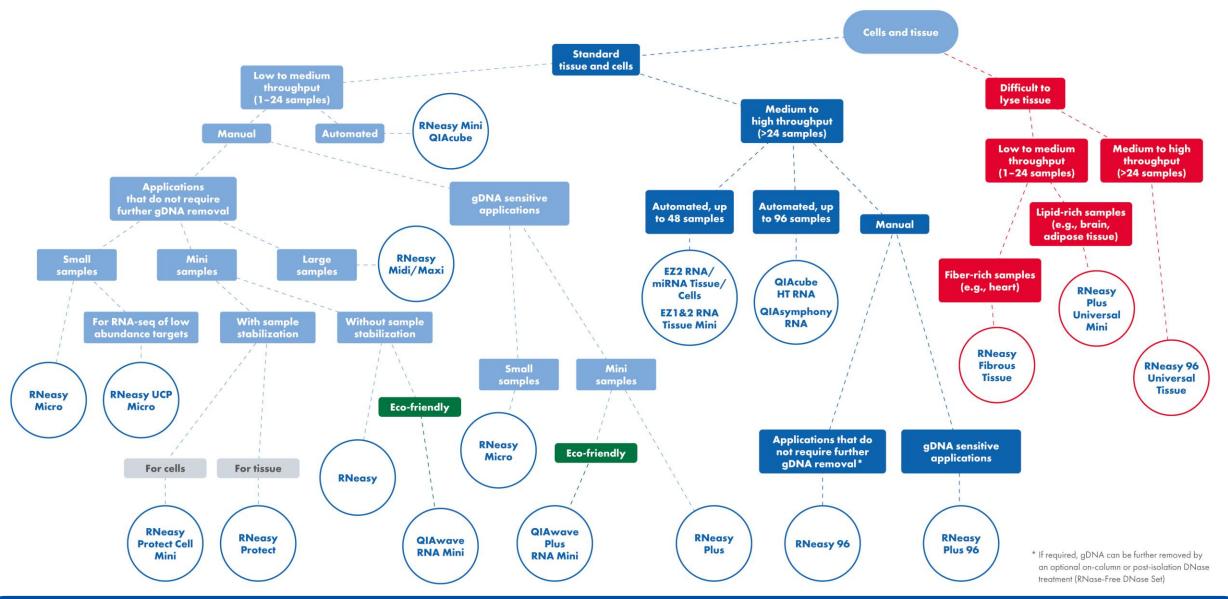
Pure, high-quality RNA

Highly efficient gDNA removal



## Choose the right kit for your cells and tissue samples





## Gene expression analysis beyond RNA sample prep



Sample collection and stabilization

Sample preparation

**Analysis: NGS,** digital PCR, qPCR

**Data analysis** 

**Biological** interpretation

Integrated RNA collection, stabilization and preparation

GeneGlobe Design & Analysis Hub

Insightful data analysis and interpretation

Total RNA sample preparation kits

Differential gene expression profiling by NGS

**RNA-seq Analysis** Portal

GeneGlobe Data

**Analysis Center** 

Explore and compare curated 'omics data

Simultaneous DNA and RNA preparation

Automated sample

preparation

Absolute gene quantification via dPCR

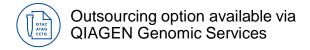
Digital PCR system

Gene expression profiling by qPCR

Sample types in focus:

- FFPE tissue
- Cells and tissue
- Whole blood
- Liquid biopsy

Automated sample quality control





## Thank you for your attention. Questions?



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