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miRNA purification





Kits for purification of total RNA, including miRNA



microRNA and total RNA purification from tissues & cells



microRNA and total RNA purification from FFPE tissue & stabilized tissue



microRNA and total RNA purification from blood, plasma and serum



microRNA and total RNA purification from exosomes



Automated microRNA and total RNA purification



Beyond sample prep – QIAGEN microRNA workflow



Selecting the right kit – miRNA isolation

Kits for purification of total RNA, including miRNA





Sample	Kit	Features	Processing	Sample amount	Format	Elution volume
Single-spin						
	miRNeasy Tissue/Cells Advanced Mini (50)	Phenol-free RNA/ miRNA (>18 nt) purification from tissue and cells	Manual/ centrifugation or QIAcube	Up to 30 mg frozen tissue (15 mg stabilized) or up to 1 x 10^7 cells	Mini column	>30 µL
	miRNeasy Mini (50)	QIAzol-based RNA/ miRNA (>18 nt) purification	Manual/ centrifugation or QIAcube	50 mg (100 mg for adipose) tissue, 1×10^7 cells	Mini column	>30 µL
Cells and tissue	AllPrep DNA/RNA/miRNA Universal (50)	High yields of DNA, RNA, and miRNA from the same sample	Manual/ centrifugation or QIAcube	Up to 1 x 10^7 cells, up to 30 mg tissue, up to 1,5 mL blood (maximum of $1x10^7$ Leukocytes)	Mini column	>30 µL
	miRNeasy Tissue/Cells Advanced Micro (50)	Phenol-free RNA/ miRNA (>18 nt) purification from small samples	Manual/ centrifugation	Up to 5 mg tissue, up to 1 x 10 ⁶ cells	Micro column	Default 20 μL, min. 10 μL
	miRNeasy Micro (50)*	QIAzol-based RNA/ miRNA (>18 nt) purification optimized for small samples	Manual/ centrifugation or QIAcube	5 mg (10 mg for adipose) tissue, 1 x 10^6 cells	Micro column	>10 µL
	RNeasy FFPE (50)	RNA/miRNA (>18 nt) isolation from FFPE tissue	Manual/ centrifugation or QIAcube	1*5 μm to 4*10μm sections	Mini column	14–30 μL
FFPE tissue and stabilized human tissue	PAXgene Tissue RNA/miRNA (50)	To be used in conjunction with PAXgene Tissue Containers, for stabilized human tissue	Manual (centrifugation)	4 x 15 x 15 mm of human tissue fixed and stabilized in PAXgene Tissue Containers	Mini column	14–40 μL
Stabilized blood	PAXgene Blood miRNA (50)	For miRNA/RNA isolation from blood stabilized in PAXgene tubes	Manual/ centrifugation or QIAcube	2.5 mL PAXgene stab. blood	Mini column	80 μL
	miRNeasy Serum/ Plasma Advanced (50)	Phenol-free RNA/ miRNA (> 18 nt) purification from small volumes of serum and plasma	Manual/ centrifugation or QIAcube	200–600 μL of serum/ plasma	Micro column	>10 µL
Serum, plasma	miRNeasy Serum/Plasma (50)	QIAzol-based RNA/ miRNA (> 18 nt) purification from small volumes of serum and plasma	Manual/ centrifugation or QIAcube	200 μL serum/ plasma	Micro column	>10 µL
Prefiltered plasma, cerebrospinal fluid, cell culture supernatants and urine for RNA/ miRNA isolation from exosomes	exoRNeasy Midi (50)	For purification of exosome-derived RNA/ miRNA (>18 nt)	Manual/ centrifugation or QIAcube	Max 1 mL serum or plasma, max. 4 mL urine, max. 2 mL CSF, max 8 mL cell culture supernatant	Midi/micro column	>10 µL
	exoRNeasy Maxi (50)	For purification of exosome-derived RNA/ miRNA (>18 nt)	Manual/ centrifugation or QIAcube	Max 4 mL serum or plasma, max. 16 mL urine, max. 8 mL CSF, max 32 mL cell culture supernatant	Midi/micro column	>10 µL

^{*}Supplementary Protocol for RNA/miRNA isolation from plants

Kits for purification of total RNA, including miRNA





Sample	Kit	Features	Processing	Sample amount	Format	Elution volume
96-well						
	miRNeasy 96 (4 x 96)	QIAzol-based RNA/miRNA purification in 96-well format	Centrifugation, QIAvac/ Centrifugation	50 mg (100 mg for adipose) tissue, 1 x 10 ⁷ cells	96-well plate	Min. 2 x 45 μL
Cells and tissue	miRNeasy 96 Tissue/ Cells Advanced (4 x 96)	Phenol-free RNA/miRNA purification in 96-well format	Centrifugation, QIAvac/ Centrifugation	Up to 30 mg frozen tissue (15 mg stabilized) or up to 1×10^7 cells	96-well plate	70-100 μL, min. 45 μL
Kits for automation	platforms					
	EZ2 RNA/miRNA Tissue/Cells (48)	Automated purification of RNA and miRNA from human and animal cells and tissues on the EZ2	Automated EZ2	Up to 30 mg (frozen) or 15 mg (stabilized) tissue or 10 mg frozen spleen or lung tissue, or up to 5 x 10 ⁶ cells	Cartridge/ mag beads	50 or 100 μL
Cells and tissue	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	Automated QIAcube HT	Up to 5 x 10^5 cells, homogenized in 140 μ L Buffer RLT	96-well plate	110 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	Automated QIAsymphony	For 2 x 96 samples: ≤3 x 10 ⁶ cultured cells, ≤20 mg easy-to-lyse tissue, ≤20 mg fibrous tissue, For 2 x 48 samples: 3 x 10 ⁶ –1 x 107 cultured cells ≤50 mg easy-to-lyse tissue	Cartridge/ mag beads	50 or 100 or 200 μL
Serum, plasma	miRNeasy 96 Advanced QIAcube HT (5 x 96)	Phenol-free miRNA isolation from human, mouse or rat serum and plasma samples	Automated QIAcube HT	Up to 200 μL human, mouse or rat serum and plasma samples	96-well plate	80–100 μL
Stabilized blood	QIAsymphony PAXgene Blood RNA (96)	Automated purification of intracellular RNA (including miRNAs) from stabilized blood	Automated QIAsymphony	2.5 ml whole blood (4.6 x $10^6 - 1.1 \times 10^7$ leukocytes/mL)	Cartridge/ mag beads	80 or 120 or 200 μL
Solution-based						
	QIAzol (200 mL)	Phenol/guanidine-based, optimized for fatty tissue	Manual	Scalable, recommended in combination with RNeasy silica membrane technology	Reagent	N.A.

miRNeasy Tissue/Cells Advanced Kits – phenol-free miRNA purification

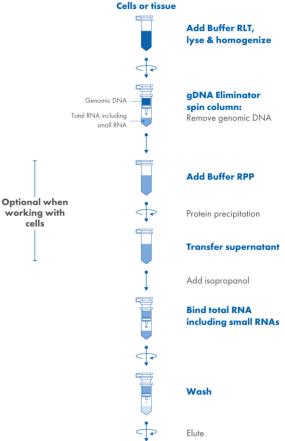


For phenol-free purification of micro-RNA and total RNA from tissues and cells

- Phenol-free alternatives to classical miRNeasy Kits – no need for phase separation or working under a hood
- Fast and convenient gDNA removal due to the advanced chemistry and gDNA Eliminator Columns/Plate
- Advanced chemistry is available in a micro-, mini or 96-well format
- Efficient enrichment of miRNA and RNAs <200 nucleotides
- Consistent, pure RNA for all downstream applications

miRNeasy Tissue/Cells Advanced Micro Kit	miRNeasy Tissue/Cells Advanced Mini Kit	miRNeasy 96 Tissue/Cells Advanced Kit
Micro column	Mini column	96-well plate
RNA <18 nt	RNA <18 nt	RNA <18 nt
Up to 5 mg tissue, up to 1 x 10 ⁶ cells	Up to 30 mg frozen tissue (15 mg stabilized) or up to 1 x 10 ⁷ cells	Up to 30 mg frozen tissue (15 mg stabilized) or up to 1 x 10 ⁷ cells
Default 20 μL, min. 10 μL	>30 µL	70–100 μL, min. 45 μL
Manual (centrifugation), automated		Manual (centrifugation or centrifugation/vacuum)
QIAcube Connect		QIAcube HT
gDNA Elimir	gDNA Eliminator Plate	
21 samples in less than 55 minutes		Varies based on processing (>36 min for centrifugation)
	Advanced Micro Kit Micro column RNA <18 nt Up to 5 mg tissue, up to 1 x 10 ⁶ cells Default 20 µL, min. 10 µL Manual (centrifug QIAcube gDNA Elimin	Micro column Mini column RNA <18 nt Up to 5 mg tissue, up to 1 x 10 ⁶ cells Default 20 μL, min. 10 μL Manual (centrifugation), automated QIAcube Connect gDNA Eliminator Columns





miRNeasy Tissue/Cells Advanced Kits – phenol-free miRNA purification





Easier handling

- No phase separation required
- Faster procedure
- Less contamination



Odorless

- · Work at the bench
- No hood required
- · No special waste management necessary



Less toxic

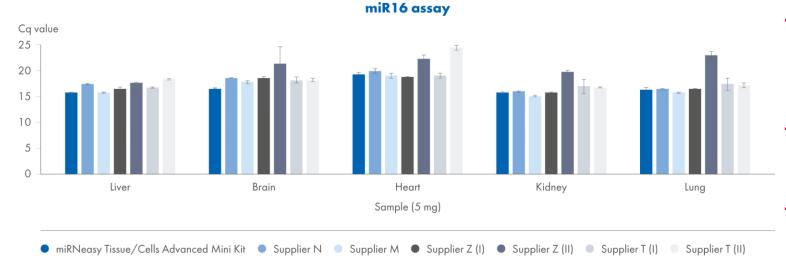
- Less exposure to health hazards
- Less harmful to your health
- Better for the environment



Prjibelski AD. et al. Accurate isoform discovery with IsoQuant using long reads. *Nat Biotechnol* 2023;41(7):915–918

Chryplewicz A,. et al. Cancer cell autophagy, reprogrammed macrophages, and remodeled vasculature in glioblastoma triggers tumor immunity. Cancer Cell. 2022;40(10):1111–1127

The miRNeasy Tissue/Cells Advanced Mini Kit shows a comparable or better performance than kits from other suppliers



- Total RNA including small RNA was isolated from 5 mg stabilized rat tissue (liver, brain, heart, kidney and lung) using the miRNeasy Tissue/Cells Advanced Mini Kit and 6 different kits from other suppliers
- Four microliters of recovered RNA, in a total volume of 20 µl using the miRCURY System, was used for miRNA detection on the miR-16
- The miRNeasy Tissue/Cells
 Advanced Mini Kit shows a
 comparable or better
 performance than kits from other suppliers

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

miRNeasy – efficient miRNA isolation including miRNA from difficult-to-lyse tissues

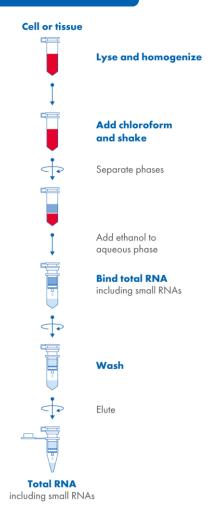


Effective microRNA (miRNA) and total RNA purification, even from small samples

- Efficient enrichment of miRNA and RNAs <200 nucleotides
- Protocols for copurification or isolation of separate fractions
- High-purity RNA suitable for all downstream applications
- Automatable protocols and high-throughput processing in 96-well format
- Combine phenol/guanidine-based lysis of samples with silica membrane-based purification

	miRNeasy	miRNeasy Mini	miRNeasy 96	
Format	Micro column	Mini column	96-well plate	
Target	RNA <18 nt	RNA <18 nt	RNA <18 nt	
Sample material	5 mg (10 mg for adipose) tissue, 1 x 10 ⁶ cells	50 mg (100 mg for adipose) tissue,1 x 10 ⁷ cells	50 mg (100 mg for adipose) tissue, 1 x 10 ⁷ cells	
Elution volume	Default 14 μL, min. 10 μL	>30 µL	Min. 2 x 45 μL	
Processing	Manual (centrifu	Manual (centrifugation or centrifugation /vacuum)		
Automation	QIAcut	-		
gDNA removal	QIAzol, additional trace amounts of gDNA can be removed via optional On-Column DNase Digestion			
Processing time	21 samples in less than 55 minutes			

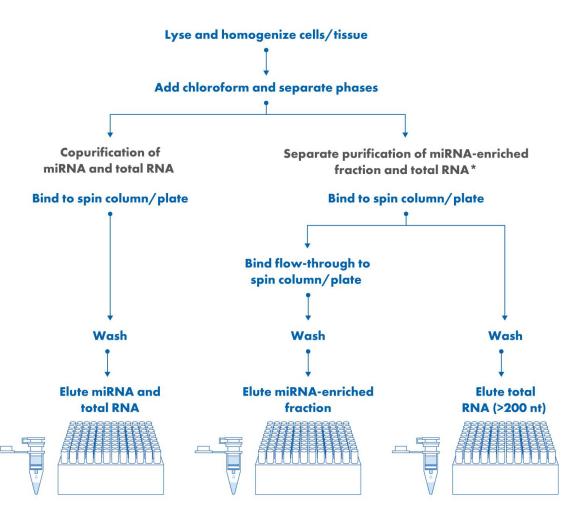
miRNeasy Mini Procedure



miRNeasy – efficient miRNA isolation including miRNA from difficult-to-lyse tissues



Either co-purify miRNA/total RNA or a miRNA-enriched fraction



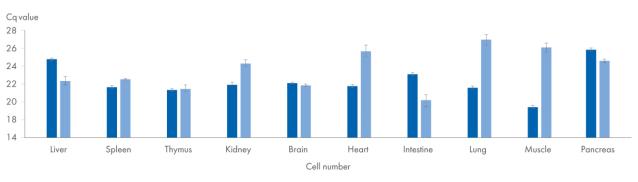
*For the recovery of the miRNA-enriched fraction, RNeasy MinElute Cleanup Kit (cat. no. 74204) is required

miRNeasy Kits outperform TRIzol regarding purity and yield



- Total RNA including miRNA was purified from a range of amounts of Jurkat cells using the miRNeasy Mini Kit or TRIzol Reagent
- Results showed that Cq values were lower after purification using the miRNeasy Kit, indicating that higher amounts of miRNA were purified than using TRIzol

Efficient copurification from miRNA & total RNA from various tissues



- miR-16 PGK1
- Total RNA including miRNA was purified from 25 mg of a range of RNAprotect Tissue Reagent stabilized rat tissues using the miRNeasy 96 Kit
- Results showed successful detection of both PGK1 mRNA (large RNA) and miR-16 (small RNA) from the same eluates

miRNeasy Advanced vs. miRNeasy

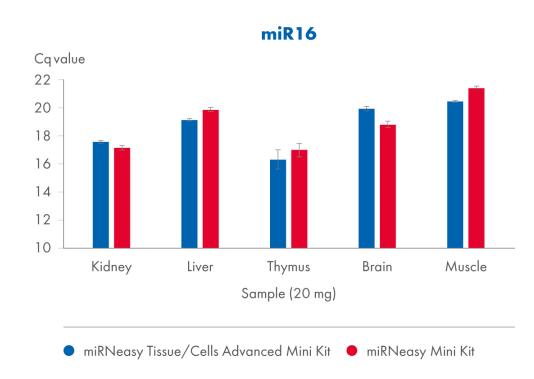


	miRNeasy/ QIAzol based kits		Phenol-free miRNeasy Advanced kits	
Environmental impact	Phenol/guanidine-based QIAzol Lysis Reagent	_ _	Less toxic, phenol-free, no special waste management required	++
gDNA removal	QIAzol, optional: On-column DNA digest	_	gDNA Eliminator Columns	+++
Preparation time	Longer procedure	_	Fast procedure due to gDNA Eliminator Column/Plate	+++
Difficult to lyse tissue (e.g., lipid tissue)	Well-suited even for difficult to lyse tissues/samples	+++	Not well suited	_
Handling	Requires phase separation, safety hood required, smelly	_ _	Easier handling, does not require phase separation, odorless	++
Popularity	More than 27,000 citations	+++	New kits, therefore, fewer citations in the scientific literature	_
Formats	Micro, mini and 96-well	+	Micro, mini and 96-well	+

Did you know?

Phenol is not only harmful to human health but also has the potential to bioaccumulate in living organisms, particularly in aquatic ecosystems. Go phenol-free to protect the environment and your health and enjoy the easier handling of the Advanced kits.

High recovery of miRNA without the need for phenol



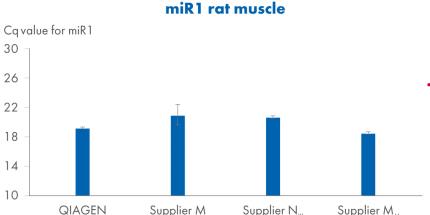
- Total RNA including small RNA was isolated from 20 mg frozen rat tissue using the miRNeasy Tissue/Cells Advanced Mini Kit (blue bars) and the miRNeasy Mini Kit (red bars)
- Both kits show comparable performance, even for difficult tissue types like muscle or brain.

AllPrep DNA/RNA/miRNA Universal Kit – simultaneous purification of gDNA and miRNA/RNA

High yields of DNA, RNA, and miRNA from the same sample

- Developed for difficultto-lyse samples (e.g., fiber- or lipid-rich tissue)
- Delivers high-quality nucleic acids, ready for downstream use
- Preoptimized protocols for various sample types
- Convenient procedure that does not require the use of phenol

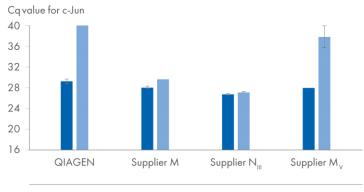
AllPrep DNA/ RNA/miRNA Universal Kit delivered higher yields of miRNA



 miR1 expression levels in muscle tissue were investigated using miRNA isolated from the AllPrep DNA/RNA/miRNA Universal Kit and products from the indicated suppliers

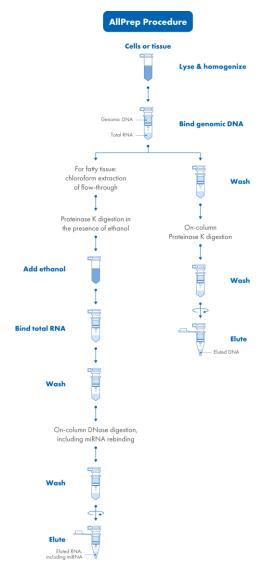
Unlike kits from other suppliers, AllPrep DNA/RNA/miRNA Universal Kit delivered high yields of pure RNA from difficult-to-lyse adipose tissue

Adipose tissue: c-Jun expression



 To visualize contamination of RNA with genomic DNA, quantitative real-time PCR assays were performed with (+RT) or without (-RT) reverse transcriptase





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

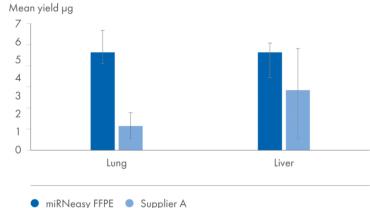
miRNeasy FFPE Kit – microRNA and total RNA purification from FFPE tissue

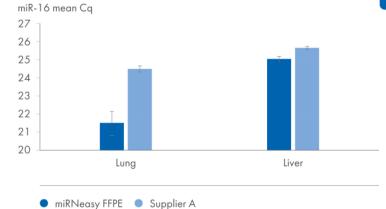


Purification of microRNA and total RNA from formalinfixed, paraffinembedded tissue sections

- Effective purification of miRNA and total RNA
- Novel method to overcome formalin crosslinking
- Efficient release of RNA without compromising integrity
- Streamlined protocol providing RNA in just 85 minutes

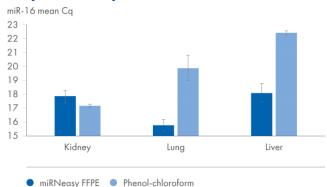
Superior yields and performance





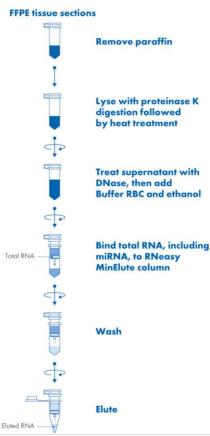
- RNA was purified from FFPE sections of the indicated rat tissues using either the miRNeasy FFPE Kit or a similar kit from Supplier A
- RNA yields were determined by measuring absorbance at 260 nm (A)
- Purified RNA was used as a template in quantitative, real-time RT-PCR assays for the miRNA miR-16 (B)

Superior to phenol-chloroform extraction



- Total RNA including miRNA was purified from the indicated rat tissues using either the miRNeasy FFPE Kit or phenol-chloroform extraction
- Purified RNA was used as a template in quantitative, real-time RT-PCR assays for the miRNA miR-16

miRNeasy FFPE Procedure



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

Stabilization of RNA/miRNA from tissue samples followed by isolation using PAXgene Tissue RNA/miRNA Kit

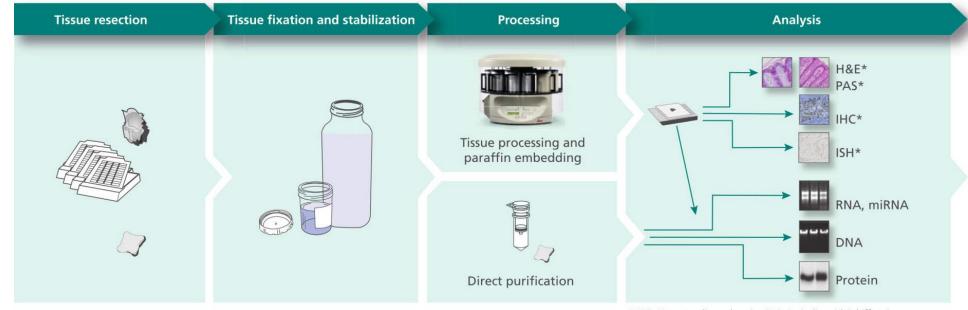




Purification of microRNA and total RNA from formalinfixed, paraffinembedded tissue sections

- Effective purification of miRNA and total RNA
- Novel method to overcome formalin crosslinking
- Efficient release of RNA without compromising integrity
- Streamlined protocol providing RNA in just 85 minutes

The PAXgene Tissue System enables simultaneous preservation of histomorphology and biomolecules



*H&E: Hematoxylin and eosin; PAS: Periodic acid-Schiff stain; IHC: Immunohistochemistry; ISH: in situ hybridization

microRNA and total RNA purification from blood, plasma and serum





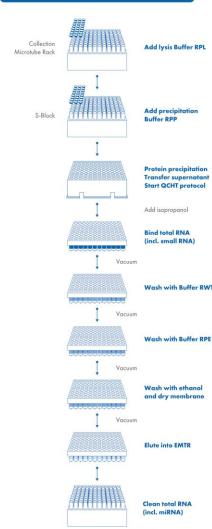
Analysis of all cellfree RNAs inside and outside of vesicles

- Optimal miRNA yields from minimal plasma amounts (200 µL)
- MinElute columns allow for small elution volumes (approx. 20 µL)
- · Consistent, pure RNA for all downstream applications
- Efficient lysis of exosomes to release exosomal miRNA

miRNeasy Serum/ Plasma Advanced	miRNeasy Serum/Plasma	miRNeasy 96 Advanced QIAcube HT (5 x 96)	Sample preparation
Micro column	Micro column	96-well plate	<20 min
RNA <18 nt	RNA <18 nt	RNA <18 nt	
No, phenol-free	Yes	No, phenol-free	Transfer 10 min
200 μL serum/plasma	200 µL serum/plasma	Up to 200 μL serum/plasma	(for 200 µL plasma samples, automated transfer possible on QIAcube HT)
No laborious phase separation or working under the hood		Convenient high-throughput automated solution on the QIAcube HT with minimal hands-on time	
10–20 μL	10–20 μL	80–100 μL	
Manual (centrifugation), automated		Automated	Sample purification on QIAcube HT 80 min
QIAcu	be Connect	QIAcube HT	
	Plasma Advanced Micro column RNA <18 nt No, phenol-free 200 μL serum/plasma No laborious phase separation or working under the hood 10–20 μL Manual (centrif	Plasma AdvancedSerum/PlasmaMicro columnMicro columnRNA <18 nt	Plasma AdvancedSerum/PlasmaQlAcube HT (5 x 96)Micro column96-well plateRNA <18 nt

- Advanced chemistry: Phenol-free alternatives to classical miRNeasy Kits
- miRNeasy Serum/ Plasma Advanced: UCP (ultra clean production) columns provide ultra-clean eluates suited for any downstream application

miRNeasy 96 Advanced QIAcube HT Procedure



miRNeasy Serum/Plasma Advanced for non-toxic miRNA isolation



Chryplewicz A, et al. Cancer cell autophagy, reprogrammed macrophages, and remodeled vasculature in glioblastoma triggers tumor immunity. Cancer Cell. 2022;40(10):1111-1127.e9

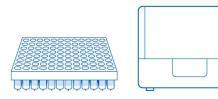


miRNeasy Serum/Plasma **Advanced Kit**



miRNeasy 96 **Advanced QIAcube HT Kit**

on QIAcube HT



High recovery of miRNA



- miRNeasy Serum/Plasma Advanced Kit
- miRNeasy Serum/Plasma Kit

miRNA was isolated from 200 µL plasma from 3 different samples using the miRNeasy Serum/Plasma Advanced Kit or the miRNeasy Serum/Plasma Kit

Both kits show good recovery of miR16

Competitive yield and performance



The miRNeasy Serum/Plasma Advanced Kit shows comparable or better performance to kits from other suppliers.

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

miRNeasy Serum/Plasma Advanced for non-toxic miRNA isolation



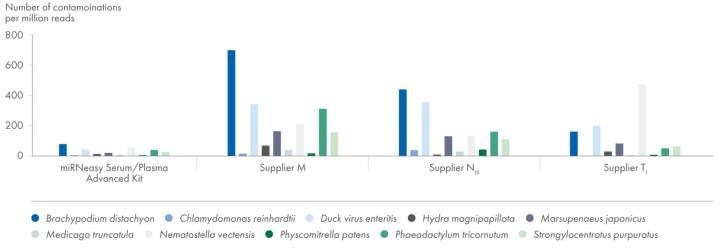
Increase your miRNA reads



Compared to miRNA samples isolated with competitor kits the miRNeasy Serum/Plasma Advanced Kit showed:

- Lowest number of "unmappable" reads
- Lowest number of "too short reads"
- Highest number of "miRNA" reads

Get better miRNA sequencing performance combined with lower apparent contamination



- UMI-collapsed number of potential contaminants using the different isolation kits
- Isolation with the miRNeasy Serum/Plasma Advanced Kit results in better miRNA sequencing performance combined with lower apparent contamination

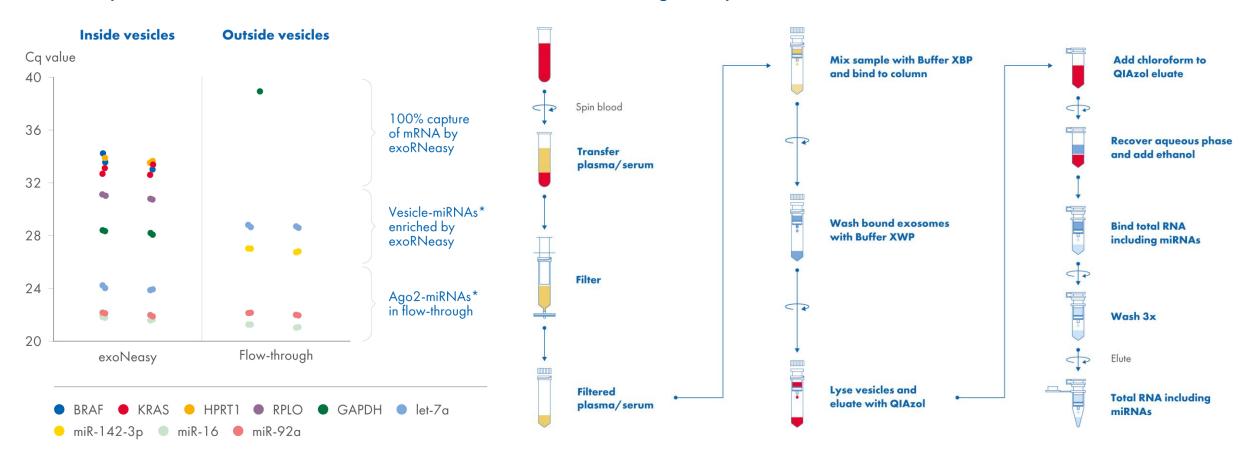
Data obtained through experiments conducted by QIAGEN R&D in Hilden, Germany.

exoRNeasy Midi and Maxi Kits – isolate all mRNA and miRNA with a standardized workflow





miRNA are present in vesicular fractions and non-vesicular fractions such as free Ago2 complexes





exoRNeasy captures all mRNA and vesicle-specific miRNA.

Automated miRNA purification





	·)))	-)))		
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	Most RNA Single-Spin kits can be processed on the QIAcube Connect. For available protocols please check here.	EZ2 RNA/miRNA Tissue/Cells (48)	QIAcube HT RNA (5x 96), QIAzol Lysis Reagent (200 mL) miRNeasy 96 Advanced QIAcube HT (5x 96)	QIAsymphony RNA QIAsymphony PAXgene Blood RNA (96)
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
Key strengths	 Automation of well-proven and established spin-columns kits for many sample materials Guided setup and UV-light decontamination of work deck for highest lab safety 	 Prefilled and sealed cartridges to minimize the risk of contamination Intuitive and easy to use 	 Allows high-throughput preparation of cell-free miRNA as well as isolation of total RNA, including miRNA from cells and tissue 	 Prefilled and sealed cartridges to minimize the risk of contamination Full tracking of samples and reagents

QIAGEN miRNA workflow





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miRNA Detection

dPCR

· QIAcuity EG

Complete offering

with RT & PCR kits,

PCR Kit

assays

QlAcuity

Sample collection/ disruption

miRNA & Exosomes Isolation

Localization ISH Northern

NGS

PCR

Data **Analysis &** Interpretation

Functional analysis

 RNAprotect Allprotect Tissue

Reagent

 PAXgene Tubes and containers

Vortex Adapters

PowerLyser 24

TissueLyser III

 miRNeasy Advanced Kits for tissue/cells, serum/plasma

exoRNeasy Kits

exoEasy Midi

 PAXgene Blood/ Tissue miRNA Systems

• EZ2 RNA/miRNA Tissue/Cells

 QIAcube Connect / QIAcube HT

EZ2 Connect

QIAsymphony SP/AS

· QIAxcel Connect (for nucleic acid quality control)

 miRCURY LNA miRNA Detection Probes

 miRCURY LNA miRNA FFPE ISH Optimization kits (Complete kits for FFPE ISH)

 QIAseg miRNA Library Kit

 QIAseq miRNA Library QC PCR Panel and Assays

(for NGS library

quality control)

QIAxcel Connect

qPCR

 miRCURY LNA miRNA PCR (SYBR Green)

 miRCURY LNA miRNA Probe PCR

 Complete systems with RT & PCR kits, assays and panels

QlAgility

QIAquant

· Rotor-Gene Q

QIAsymphony SP/AS

· GeneGlobe Data **Analysis Center**

 Ingenuity Pathway Analysis

 CLC Genomics Workbench

OmicSoft

 miRCURY LNA miRNA Mimics. Inhibitors, and Target Site Blockers for functional analysis in vitro and in vivo

 HiPerFect transfection kit

• QIAGEN Genomic Services: Sample preparation, NGS analysis, dPCR and gPCR analysis, data analysis and biological interpretation

Services

Assays

Kits &

Instruments

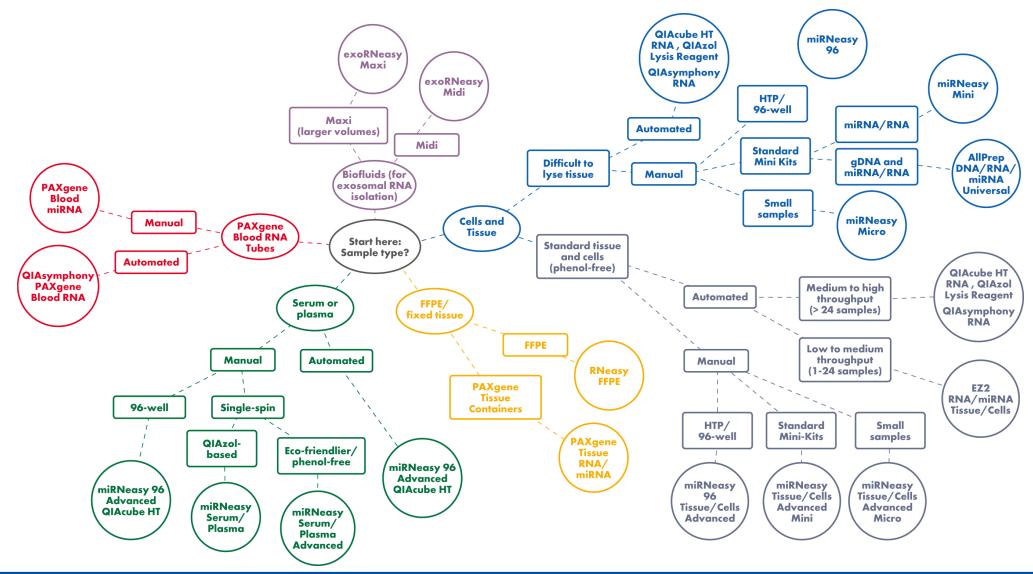
miRNA extraction is the first out of many workflow steps leading to new insights.

A quick guide to help you choose the right kit for your miRNA isolation experiment





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Thank you for your attention. Questions?



Trademarks: QIAGEN®, Sample to Insight®, QIAcube®, QIAcuity®, QIAsymphony®, QIAxcel®, QIAgility®, QIAquant®, QIAzol®, AllPrep®, EZ2®, GeneGlobe®, Ingenuity®, IPA®, LNA®, miRCURY®, RNeasy®, Rotor-Gene® (QIAGEN); PAXgene® (PreAnalytiX GmbH). Registered names, trademarks, etc. used in this document, even when not specifically marked as such, may still be protected by law. QPRO-6452 © 2024 QIAGEN, all rights reserved..