

## Product Profile

# QIAamp® Circulating Nucleic Acid Kit

For concentration and purification of free-circulating DNA and RNA from human plasma or serum

The QIAamp Circulating Nucleic Acid Kit greatly simplifies the isolation of circulating DNA and RNA from plasma or serum. Nucleic acids are efficiently purified and concentrated from starting materials that contain low concentrations of mostly fragmented DNA and RNA (typically 1–100 ng/ml circulating DNA in human plasma).

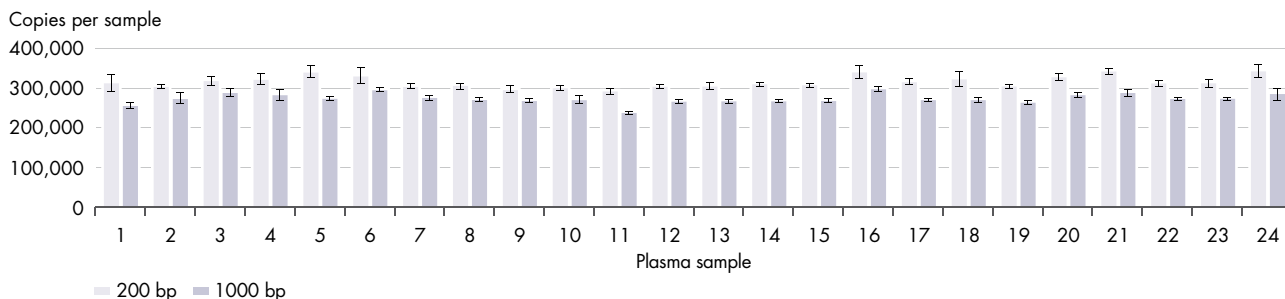
The QIAamp Circulating Nucleic Acid Kit provides:

- Concentrated nucleic acids, with high input and low elution volumes
- Efficient recovery of fragmented DNA and RNA
- No organic extraction or ethanol precipitation
- Complete removal of contaminants and inhibitors

## Advanced technology for efficient recovery of fragmented DNA and RNA

Analysis of tumor-specific extracellular DNA fragments and mRNAs in the blood can enable specific detection of tumor types from a simple blood sample. These circulating nucleic acids are present in serum or plasma usually as short fragments, <1000 bp (DNA) or <1000 nt (RNA). In addition,

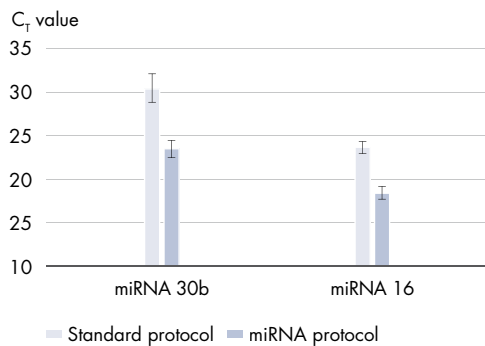
miRNAs, as small as 21 nt, have the potential to provide biomarkers for certain cancers and disease states. The QIAamp Circulating Nucleic Acid Kit enables efficient purification of these circulating nucleic acids from human plasma or serum and other cell-free body fluids (Figure 1). ▷



**Figure 1. Reproducible recovery of circulating DNA.** DNA fragments (200 bp and 1000 bp) in equal amounts were added to 24 plasma samples. DNA was purified from 5 ml plasma using the QIAamp Circulating Nucleic Acid Kit, with an elution volume of 80 µl. DNA yield was quantified by duplex, real-time PCR of 66 bp targets specific for each DNA fragment using the QuantiTect Multiplex PCR Kit.



**Figure 2. QIAamp Mini columns with tube extenders for processing on the QIAvac 24 Plus.**

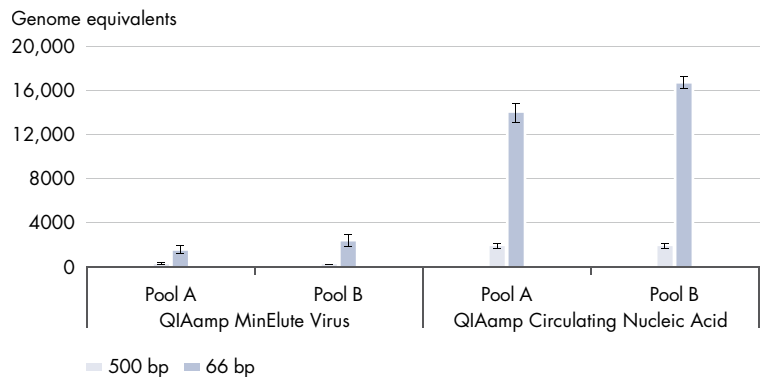


**Figure 4. Specialized protocol for efficient purification of circulating miRNA.** Circulating RNA was purified from 4 ml pooled plasma using the QIAamp Circulating Nucleic Acid Kit, with an elution volume of 50  $\mu$ l. The standard kit protocol was compared with the specialized protocol for miRNA. miRNA 30b yield was quantified using a TaqMan<sup>®</sup> microRNA assay (Applied Biosystems<sup>®</sup>), and miRNA 16 yield was quantified using a Human miScript Assay (QIAGEN). The lower  $C_t$  values with the miRNA protocol indicate higher yields of the miRNA species.

Tube extenders and vacuum processing on the QIAvac 24 Plus enable starting sample volumes of up to 5 ml (Figure 2), and flexible elution volumes between 20  $\mu$ l and 150  $\mu$ l allow concentration of nucleic acid species that are present in low concentrations.

## Efficient recovery of fragmented nucleic acids

The kit provides advanced technology of selective binding to a silica-based membrane for improved recovery of fragmented nucleic acids (Figure 3). Purification is highly efficient, with reproducible yields, so as to provide a representative population of the circulating nucleic acids in blood (Figure 1).



**Figure 3. Improved recovery of fragmented DNA.** Circulating DNA was purified from 5 ml plasma using the QIAamp Circulating Nucleic Acid Kit or from 1 ml plasma using the QIAamp MinElute<sup>®</sup> Virus Vacuum Kit, with elution volumes of 100  $\mu$ l. DNA yield was quantified by duplex, real-time PCR of a 66 bp amplicon and a 500 bp amplicon within the 18S rRNA gene using the QuantTect<sup>®</sup> Multiplex PCR Kit. The difference between amplification of the 500 bp amplicon and 66 bp amplicon shows that the DNA is fragmented, resulting in a lower abundance of intact 500 bp target sequences compared with the shorter 66 bp target. (Note that the difference is greater than the fivefold difference in sample input volumes.)

## Purification of circulating nucleic acids, including miRNAs and methylated DNA

Purification of circulating RNA, without copurification of DNA, is possible by optional on-column DNA digestion using the RNase-Free DNase Set. A specialized protocol provides highly efficient purification of small RNA, such as miRNAs (Figure 4).

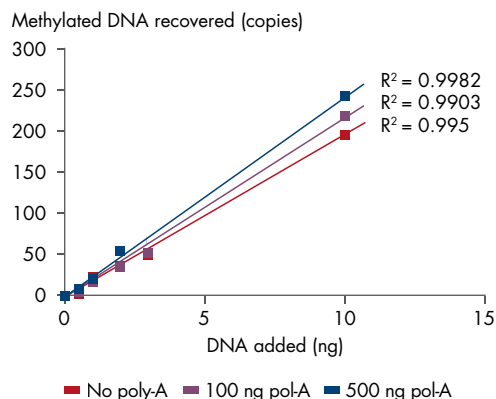
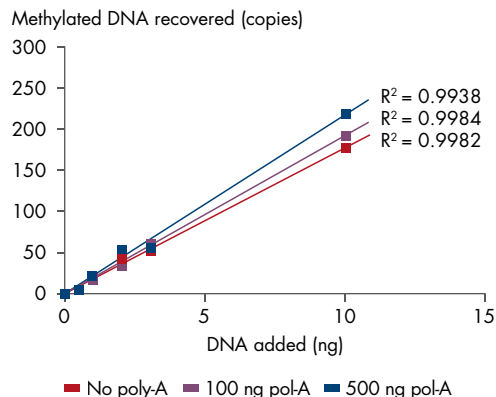
Methylated DNA can be efficiently purified using the QIAamp Circulating Nucleic Acid Kit. The purified DNA maintains its methylation, allowing bisulfite conversion for analysis of the methylation state (Figure 5).

## Pure, concentrated DNA and RNA for sensitive downstream applications

The purified and concentrated circulating DNA and RNA is free of proteins, nucleases and other impurities, and is ready to use in wide range of downstream applications, including:

- PCR and quantitative real-time PCR and RT-PCR
- Biomarker research and validation for blood-based cancer detection
- Viral nucleic acid detection

**Figure 5. Efficient recovery of methylated DNA.** Spiked methylated DNA and non-target poly-A RNA were purified from 4 ml plasma using the QIAamp Circulating Nucleic Acid Kit, with an elution volume of 45  $\mu$ l. Bisulfite conversion was carried out using the EpiTect<sup>®</sup> Bisulfite Kit (QIAGEN), and methylation-specific PCR (MSP) was performed using real-time MSP assays and components of the QuantiTect Multiplex PCR Kit on the ABI PRISM<sup>®</sup> 7900HT Sequence Detection System. Data from two fully methylated loci were analyzed.



### Kit specifications

Sample volume	1–5 ml
Elution volume	20–150 $\mu$ l
Processing time (standard protocol)	<2 hours (for 24 samples)
Nucleic acid fragments recovered	>20 bp DNA, >20 nt RNA

## Ordering Information

Product	Contents	Cat. no.
QIAamp Circulating Nucleic Acid Kit (50)	For 50 preps: QIAamp Mini Columns, Tube Extenders (20 ml), QIAGEN Proteinase K, Carrier RNA, Buffers, VacConnectors and Collection Tubes (1.5 ml and 2 ml)	55114
QIAvac 24 Plus	Vacuum manifold for processing 1–24 spin columns: QIAvac 24 Plus Vacuum Manifold, Luer Plugs, Quick Couplings	19413
RNase-Free DNase Set (50)	1500 units RNase-free DNase I, RNase-free Buffer RDD and RNase-free water for 50 RNA minipreps	79254

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