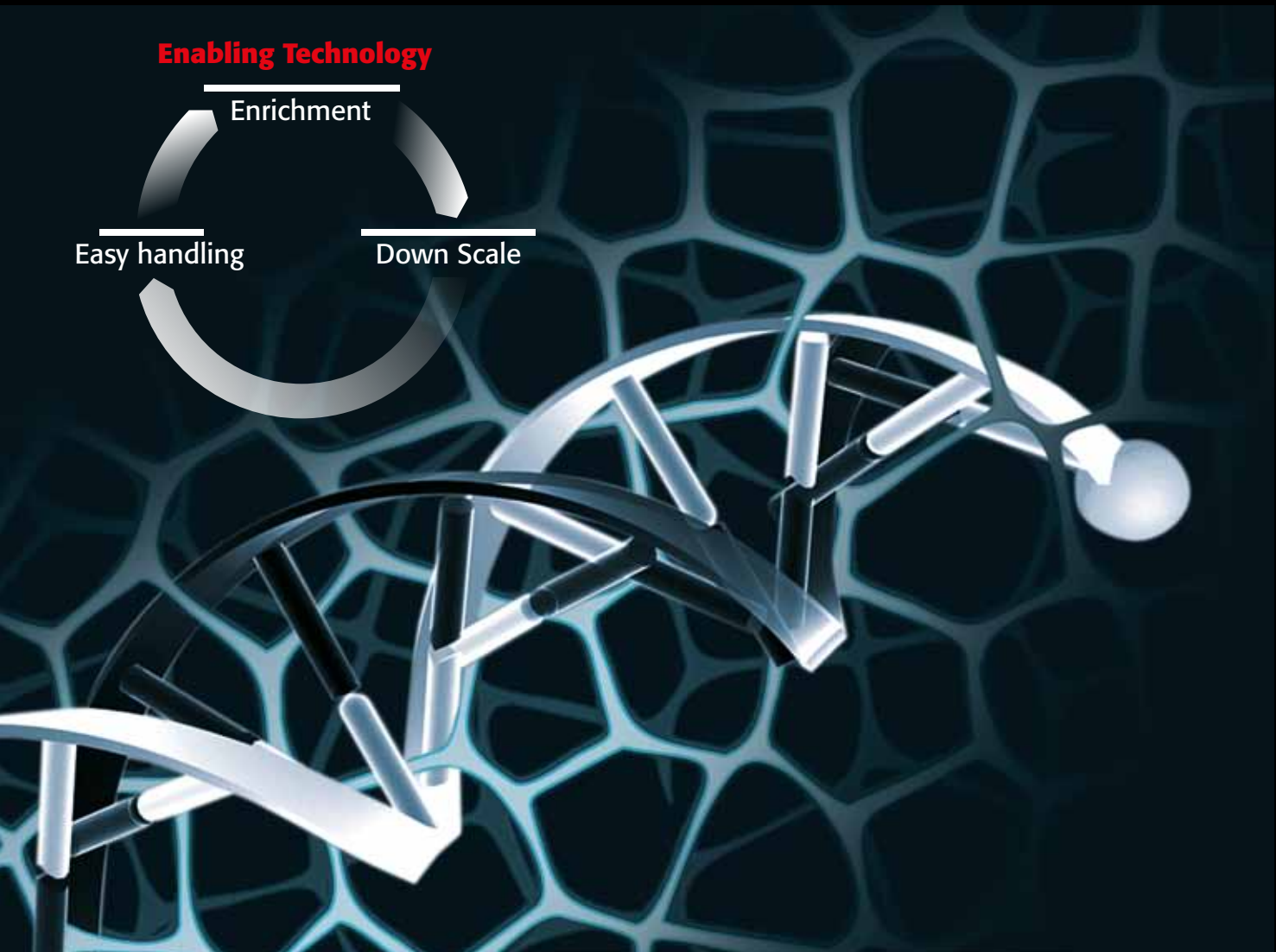
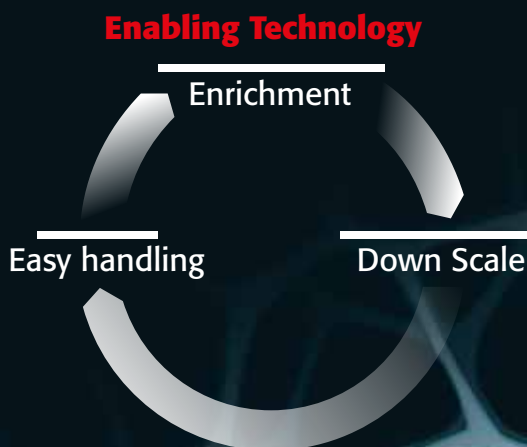


E_{asy}fficient free-circulating DNA extraction

Polymer-mediated enrichment opens up a whole new world

- Extremely easy-to-handle, highly efficient method that saves considerable time
- Processes sample volumes of up to 10 mL
- Positive test results in urine (up to 10 mL), serum and plasma from different blood samples



PME free-circulating DNA Extraction Kit



Product description

Regardless of whether a metabolic disorder, prenatal diagnostics or sports medicine is at issue—when it comes to reliable diagnostic results, the chances of a positive outcome are better the earlier and more precise those results are. Thanks to its PME free-circulating DNA Extraction Kit, Analytik Jena AG now offers an innovative product for an increasingly important diagnostic method. Examining circulating, cell-free DNA is particularly likely to yield valuable information in the field of modern tumor diagnostics, where that information can be used for early detection and for gauging the progression of cancers.

The kit is based on novel **PME** (Polymer-Mediated Enrichment) technology (patent pending), an incomparably efficient method for enriching and isolating freely circulating DNA. Even when the amount of freely circulating DNA is very small, and only small nucleic acid fragments (< 1000 nt) are available, this kit delivers fast, reliable results – using only a minimal amount of reagent.

The first step utilizes a polymer for binding cell-free DNA from the full sample, after which the complex is pelletized via centrifugation. The final step involves dissolving the bound nucleic acid in a special buffer, which significantly reduces the sample volume for the subsequent extraction.

Product specifications

Starting material:

- Serum and plasma (up to 5 ml)
- Cell culture supernatants or mediums (up to 5 ml)
- Other cell-free body fluids (up to 5 ml)
- Urine samples (up to 10 ml)

Extraction time:

- From 1 ml Samplevolume: appr. 30 min
- From 2 ml - 10 ml samplevolume: appr. 1 h

Field of applications:

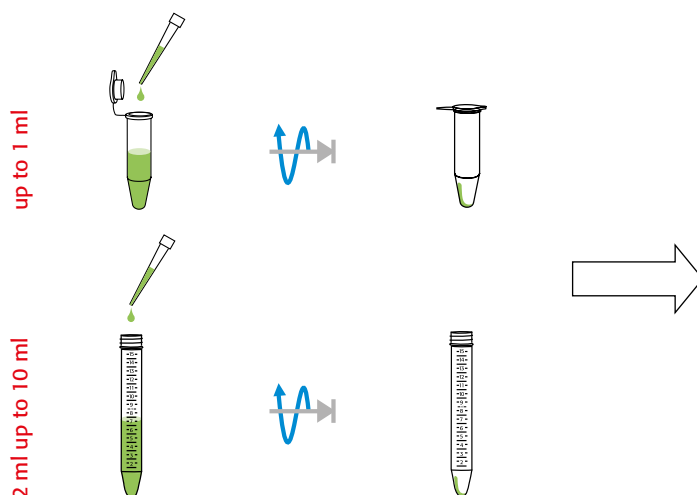
- Tumor and prenatal diagnosis
- Pathological states, including trauma, sepsis, myocardial infarction, stroke, transplantation, diabetes mellitus and hematologic disorders

Validation

Positive tested for following blood collection systems:

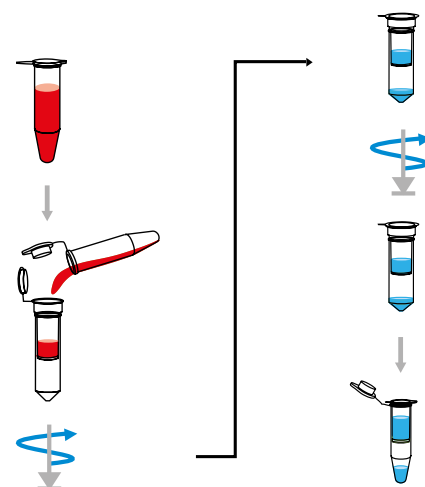
No.	Blood sampling system from Sarstedt
1.	S-Monovette® 9 ml Silicat
2.	S-Monovette® 9 ml Polyacrylester Gel
3.	S-Monovette® 8.5 ml CPDA
4.	S-Monovette® 9 ml K3E (EDTA K ₂)
5.	S-Monovette® 10 ml 9NC (Trisodium Citrate Solution, Citrate Solution)
6.	S-Monovette® 7.5 ml NH (Natrium-Heparin)
7.	S-Monovette® 7.5 ml LH-Gel (Lithium-Heparin)
8.	S-Monovette® 9 ml LH (Lithium-Heparin)

Enrichment of free-circulating DNA



1. Capturing of cell-free DNA in the polymer
2. Spin down of the Polymer/DNA complex

Sample preparation of free-circulating DNA



3. Lysis of the Polymer/DNA complex
4. Binding of the cell free DNA to the spin filter
5. Washing of the bound cell free DNA
6. Eluting of the cell free DNA

Figure 1: General procedure of enrichment and isolation of free-circulating DNA.

Validation results/Sample application

1. Different blood collecting systems for extraction of free-circulating DNA:

Besides the variable amount of free-circulating DNA of different individuals, also the blood collection systems have a big influence on the recovery of free-circulating DNA. Therefore the above mentioned blood collection systems were tested:

Testing the suitability of the PME free-circulating DNA Extraction Kit for eight different blood collection systems (listed above), at two different starting amounts of sera or plasma (1 ml and 5 ml, respectively).
Extracted free-circulating DNA has been tested by amplification of a human specific target gene:

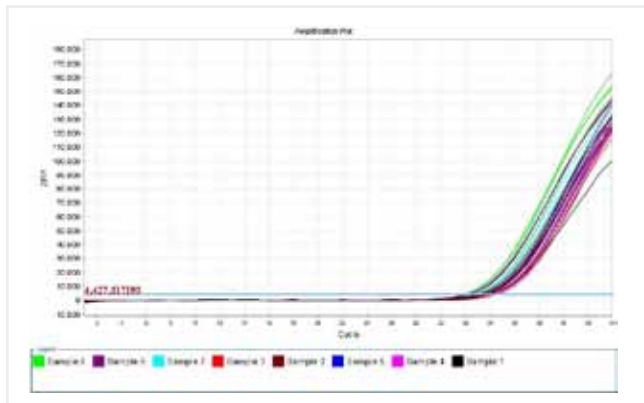


Figure 2: Resultant amplification plots after preparation of 1 ml starting sample volume

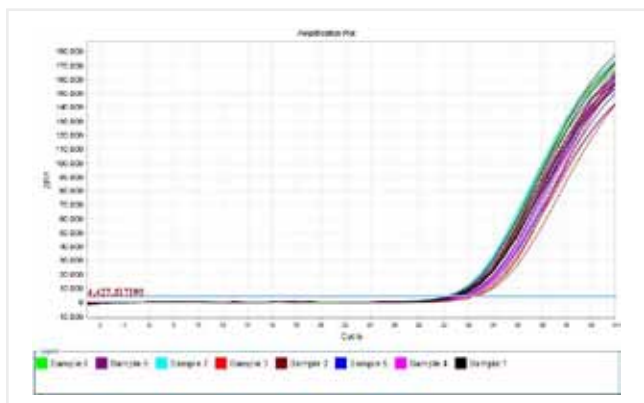


Figure 3: Resultant amplification plots after preparation of 5 ml starting sample volume.

The amplification plots show differences in dependence on type of blood collection systems. Best results can be achieved using S-Monovette® 9 ml LH (Lithium-Heparin, Sarstedt) or S-Monovette® 7.5 ml NH (Natrium-Heparin, Sarstedt) and S-Monovette® 7.5 ml LH-Gel (Lithium-Heparin, Sarstedt).

2. Isolation of free-circulating DNA using PME free-circulating DNA Extraction Kit in comparison to standard purification kit for cell-free DNA:

Next to the speed of performance and efficiency of the PME free-circulating DNA Extraction Kit, the whole procedure also convince in relation to the market leader product, as shown in the following:

Comparison of cell-free DNA extraction from 1 ml and 5 ml serum respectively by using PME technology versus a commercially available gold-standard kit for free-circulating nucleic acids. After isolation, the DNA has been tested for amplification of a gene coding for the human estrogen receptor 1:

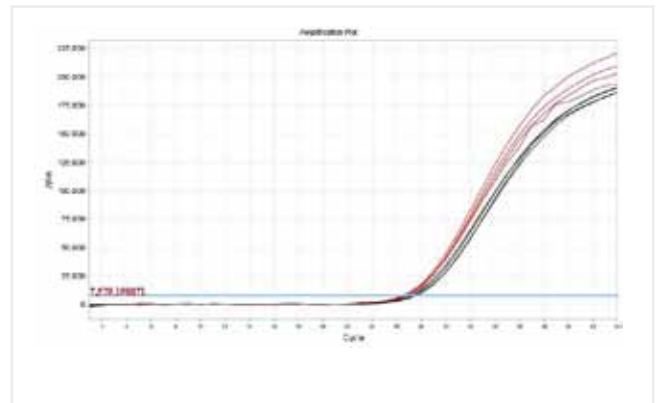


Figure 4: Resultant amplification plots after preparation of 1 ml starting sample volume

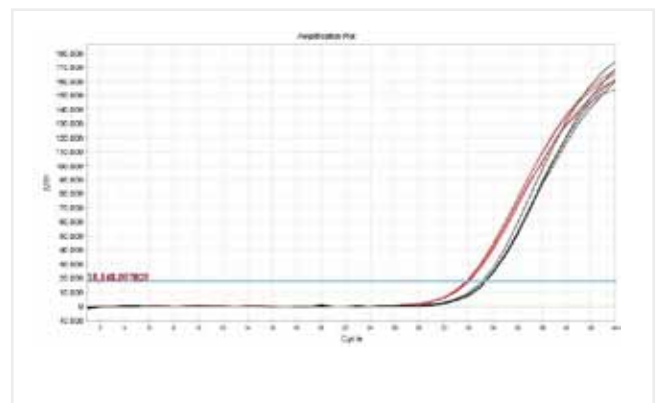


Figure 5: Resultant amplification plots after preparation of 5 ml starting sample volume

The red graphs correspond to the extraction based on PME technology and the black graphs correspond to the competitor's kit (market leader).

3. Enrichment of free-circulating DNA from 5 and 10 ml human urine sample:

Next to plasma and serum also urine samples can be processed using PME free-circulating DNA Extraction Kit. A starting volume of up to 10 ml is realized, making sure, that the final concentration of cell-free DNA is sufficient for downstream applications.

Free-circulating DNA from 5 ml and 10 ml human urine sample was extracted by using the PME free-circulating DNA Extraction Kit: Subsequently the cell-free DNA was tested and compared with DNA, extracted from maximum processable urine sample (4ml) by competing extraction kit for free-circulating nucleic acids (market leader), in real-time PCR by amplifying a human specific coding gene.

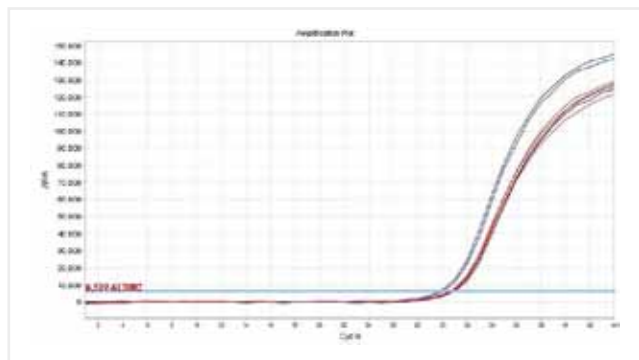


Figure 6:
The blue and black graphs correspond to the extraction from 10 ml sample and from 5 ml sample by the PME technology respectively and the red graphs correspond to the 4 ml sample by competitor's product.

Order information

PME free-circulating DNA Extraction Kit

Order number	Quantity
845-IR-0003010	10 reactions
845-IR-0003050	50 reactions

Related Products

innuCONVERT Bisulfite Basic Kit

Order number	Quantity
845-IC-1000008	8 reactions
845-IC-1000040	40 reactions
845-IC-1000080	80 reactions

innuCONVERT Bisulfite All-in-One Kit

Order number	Quantity
845-IC-2000008	8 reactions
845-IC-2000040	40 reactions
845-IC-2000080	80 reactions

innuMIX qPCR MasterMix Probe

Order number	Quantity
845-AS-1200100	100 reactions
845-AS-1200200	200 reactions

innuMIX qPCR MasterMix SyGreen

Order number	Quantity
845-AS-1300100	100 reactions
845-AS-1300200	200 reactions

innuTaq HOT-A DNA Polymerase [5 U/μl]

Order number	Quantity
845-EZ-3000500	500 Units

50x inNucleotide Mix (12.5 mM)

Order number	Quantity
845-AS-9000100	2 x 0.5 ml

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as well as further technical development!

