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Smarter Nucleic Acid Sample Preparation on ep*Motion*[®] Instruments

Automated high-throughput DNA/RNA isolation



Sample preparation kits for the isolation of:

- genomic DNA
- bacterial DNA
- viral DNA and RNA

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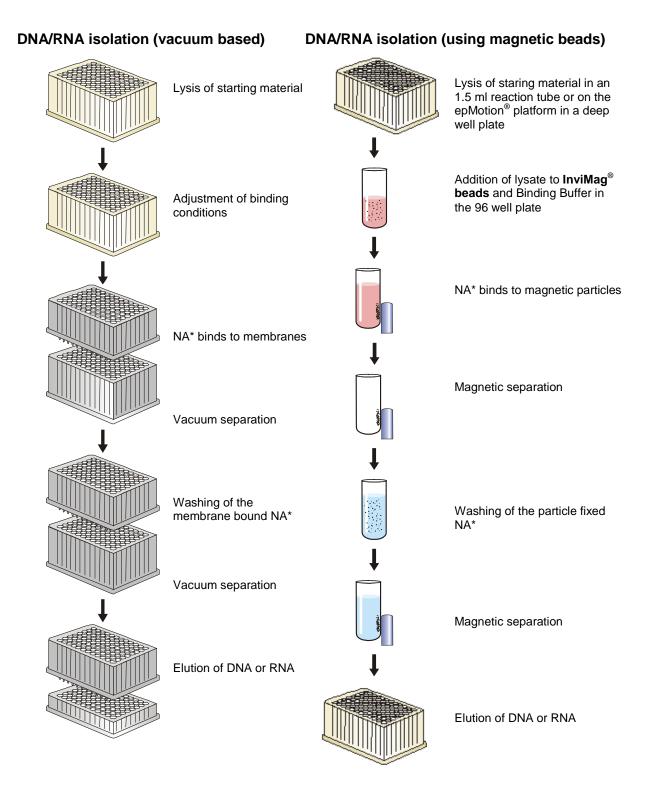
The Eppendorf ep*Motion[®]* provides an innovative concept for the automation of nucleic acid preparation. The ep*Motion[®]* and the Plug'n'Prep[®] concept enables for the first time ready to go automation on a flexible and open liquid handling platform.

The ep*Motion*[®] 5075 VAC comes equipped with 11-position deck plus one fully integrated vacuum station for filter plates. The vacuum station is self-adjusting, meaning you no longer have to "stack" collars of varying sizes around the lower plate. In addition to the range of pre-tested Plug'n'Prep[®] protocols further downstream applications can be composed individually.

In perfect combination with purification technologies from STRATEC Molecular, the ep*Motion*[®] 5075 VAC is a smart design for processing vacuum-based, mid-throughput systems for nucleic acid isolation in a 96 well format. The pipetting system provides all features for fast and convenient isolation of nucleic acids in convenient, rapid and reproducible manner – from virtually any source, such as whole blood, small tissue samples, cells, serum, plasma, swabs, forensic samples or bacteria and viruses

The ep*Motion*[®] 5075 uses low maintenance pipetting tools to move the sample through the various purification phases – binding, mixing, washing and elution. Eliminating the liquid handling and increasing automation results in a reliable and robust technique. The overall efficiency allows the ep*Motion*[®] 5075 VAC to purify 96 samples in around 70 minutes using the STRATEC Molecular technology for binding of DNA and RNA to membranes. Equally important, sample cross contamination and reagent cross-over is effectively eliminated by this automated purification process.

Isolation principles



(coming soon)

 $^{*)}$ NA = nucleic acid

Plasmid DNA isolation (vacuum based)

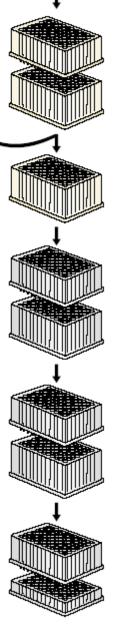
Alkaline lysis

Preclearing Removal of cell debris



Adjustment of binding conditions

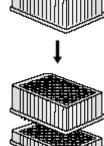
Binding of pDNA



DNA fragment purification (vacuum based)

Adjustment of binding conditions

Binding of DNA fragments



Elution of DNA fragments

Washing steps

Elution of pDNA

Product selection and application guide

Nucleic acid	Starting material	Product name	Article number	Package size
pDNA isolation	0.5 - 2.0 ml bacterial suspension	Invisorb [®] Plasmid HTS 96 Kit/ ep	7110320200 7110320300 7110320400	2 x 96 preps 4 x 96 preps 24 x 96 preps
DNA fragment purification	up to 200 µl of amplification reaction volume (PCR products from 80 bp up to 30 kb)	MSB [®] HTS PCRapace/ ep	7121240200 7121240300 7121240400	2 x 96 preps 4 x 96 preps 24 x 96 preps
	up to 200 µl blood (EDTA, citrate) up to 30 µl buffy coat, up to 20 µl bone marrow up to 25 µl non mammalian blood	Invisorb [®] Blood Mini HTS 96 Kit/ ep	7131320200 7131320300 7131320400	2 x 96 preps 4 x 96 preps 24 x 96 preps
genomic DNA isolation	lymphocyte pellet from up to 2 ml blood (EDTA, citrate)	Invisorb [®] Blood Midi HTS 96 Kit/ ep	7131720200 7131720300 7131720400	2 x 96 preps 4 x 96 preps 24 x 96 preps
	800 µI SalivaGene [®] DNA stabilizer in swab collection tube	PSP [®] SalivaGene DNA HTS 96 Kit/ ep	7136260200 7136260300 7136260400	2 x 96 preps 4 x 96 preps 24 x 96 preps
bacterial DNA isolation	bacterial pellets up to 100 µl whole blood up to 5-10 mg tissue up to 100 µl cell free body fluids (serum, plasma, synovial liquid, urine) swabs	Invisorb [®] Universal Bacteria HTS 96 Kit/ep	7138320200 7138320300 7138320400	2 x 96 preps 4 x 96 preps 24 x 96 preps
viral RNA isolation	up to 200 µl serum, plasma or other cell free body fluids up to 200 µl rinse liquid from swabs small stool sample (50 µl)	Invisorb [®] Virus RNA HTS 96 Kit/ ep	7143320200 7143320300 7143320400	2 x 96 preps 4 x 96 preps 24 x 96 preps
viral DNA isolation	up to 200 µl cell free body fluids, like serum or plasma up to 200 µl whole blood up to 200 µl rinse liquid from swabs	Invisorb [®] Virus DNA HTS 96 Kit/ ep	7142320200 7142320300 7142320400	2 x 96 preps 4 x 96 preps 24 x 96 preps

Product selection and application guide

Yield and ratio	Processing time	PCR	RT-PCR	Real-time PCR	RFLP - Analysis	RE Digestion	Southern Blot	HLA - Typing	STR - Analysis	SNP - Analysis	AFLP, RAPD - Analysis
10 - 25 μg per well	approx. 45 min	x			x	x					
80 - 95 % recovery rate	20 min for 2 x 96 reactions	x				x					
2 - 8 μg DNA depending on age, storage & source of the blood sample A ₂₆₀ :A ₂₈₀ : 1.7 - 2.0	70 min	x		X	x	x	x	x	x	x	x
8 - 20 μg DNA depending on age, storage & source of the blood sample A ₂₆₀ :A ₂₈₀ : 1.7 - 2.0	70 min after lysis	x		x	x	x	x	x	x	x	x
depending on type, source & amount of the sample	70 min	x		X	X	X		x	x	x	x
depending on type, age, source and amount of the sample A ₂₆₀ :A ₂₈₀ : 1.7 - 2.0	70 min	x		X	X	x	x		x	X	x
depending on viral load limit of sensitivity: 500 RNA virus copies/ml	70 min after lysis	x	x	x		x			x	x	
depending on viral load limit of sensitivity: 500 DNA virus copies/ml	70 min after lysis	x		x	x	x					

General purification procedure

First the samples are lysed in an optimized lysis buffer according to kit instructions. The lysis can be performed on the ep*Motion*[®] 5075 platform which allows the sample incubation due to the integrated heating block in the instrument. All subsequent steps, such as binding of the nucleic acid onto the membrane, washing and final DNA/RNA elution are automatically performed on the ep*Motion*[®] 5075 platform in a fast and reproducible manner. The highly pure nucleic acids are ready to use for downstream applications like PCR, quantitative PCR, real-time PCR or other routine methods.

Advantages

- high-speed fully automated system
- high throughput up to 96 samples per run
- cross contamination free processes
- purification of high-quality, ready to use nucleic acids
- ready made and customized purification protocols
- high binding capacity and recovery values of membranes

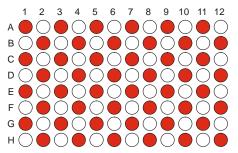
Implementation

- special customer tailored solutions are also available on request
- programs can simply be downloaded from <u>www.invitek.de</u>
- requirement: Software 2.205 or higher
- for further information please contact STRATEC Molecular: +49 30 9489 2901 or info.berlin@stratec.com

Purification of plasmid DNA from overnight culture

1. PCR inhibitor- and cross contamination-free isolation of plasmid DNA

To maximize the detection of any potential contamination event during the automated process of plasmid DNA isolation from 2 ml overnight culture of E.coli DH5 α a cross contamination assay was performed. This also ensures precise and reliable pipetting within each well without affecting adjacent wells. Positive and no template controls (only water) were arranged in alternating wells in a "chessboard" pattern illustrated in **Fig. 1**.



After DNA extraction with the **Invisorb[®] Plasmid HTS 96/ ep** the eluted samples were used for an restriction digestion with *EcoRI*. All samples were subjected to agarose gel electrophoresis analysis for detecting any contaminating DNA in the negative samples (**Fig. 2**.). No plasmid DNA was detected in the control wells with water.

1 2 3	4 5 6	7 8 9	10 11 1	.2 13 14 15

Fig. 2: Gel picture of 'chessboard pattern' of mid-section lanes, with unrestricted and EcoR1-restricted samples.

2. Sequencing

Sequencing of a pGEM plasmid from DH5 α was successfully performed with a T7-primer.

750 760 770 780 790 800 `CGGTGTAGGTCGTTCGCTCCAAGCTGGGGCTGTGTGCACGAACCCCCGTTCAGCC

Fig. 3: Sequence picture of extracted pGEM plasmid with T7-primer in "long-run"-mode from bp 750 - 800.

Purification of PCR products and DNA fragments

1. Cross contamination-free purification of DNA fragments

To maximize the detection of any potential contamination event, positive and no template controls (only water) were arranged in alternating wells. **Fig. 4** shows a gel picture with the eluted DNA (5 μ I) from 24 DNA containing samples and 24 negative samples analyzed on 1% TAE agarose gel stained with ethidium bromide. The first lane of each run shows the DNA containing sample before the purification, the second lane the DNA after purification and each third lane the no template control.

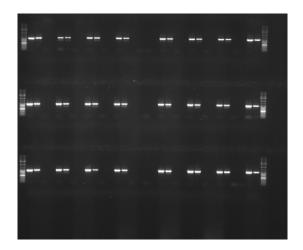


Fig. 4: 5 μ l of indicated samples without purification, 5 μ l of indicated samples with purification and no template controls with and without purification were analyzed on an agarose gel. No cross contamination has been observed.

2. Automated DNA fragment purification with high recoveries

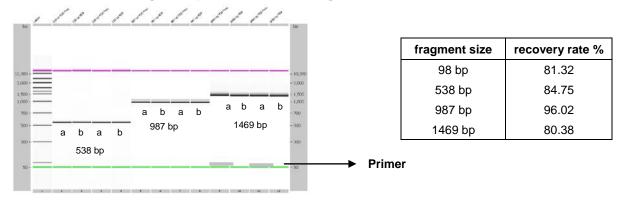


Fig. 5: Recovery Analysis with Agilent Bioanalyzer 2100. The figure shows the electropherogram for the fragments 538 bp, 987 bp and 1469 bp PCR products before (a) and after (b) **MSB[®] HTS PCRapace/ ep** purification in duplicates.

3. Automated reproducible DNA fragment purification

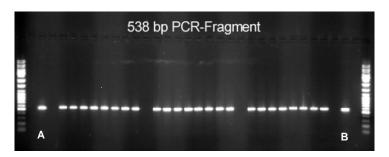


Fig. 6: A 538 bp PCR fragment was purified on the robotic platform with high and reproducible recoveries. 5 µl of the eluate were analyzed on an agrose gel. Lanes A and B show the unpurified PCR fragment.

1. PCR inhibitor- and cross contamination-free isolation of genomic DNA

To assess if the automated process of genomic DNA isolation from human blood ensures precise and reliable pipetting within each well without affecting adjacent wells a cross contamination assay was performed. Every second well was filled with water instead of the whole blood sample to create a chessboard pattern. After DNA extraction with the **Invisorb[®] Blood Mini HTS 96 Kit/ ep** all eluted samples were subjected to agarose gel electrophoresis analysis and real-time PCR as a very sensitive method for detecting any contaminating DNA in the negative samples. The isolated genomic DNA is shown in **Fig. 7**. No genomic DNA was detected in the control wells with water. The results of the PCR amplification using the same samples are shown in **Fig. 8**.

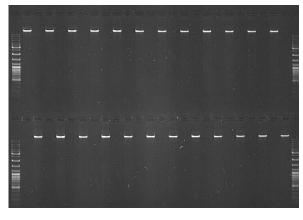


Fig. 7: The eluted genomic DNA (10 μ l) from 24 whole blood samples and 24 negative samples were analyzed on 1% TAE agarose gel stained with ethidium bromide.

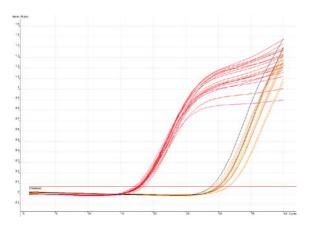


Fig. 8: The GAPDH sequence was amplified in a realtime PCR with 48 samples of the chessboard pattern (24 blood samples, red lanes and 24 no sample controls, orange lane). (Please see "Note" at page 11)

2. Automated reproducible DNA isolation

As illustrated in **Fig. 9** genomic DNA from various blood samples was isolated using the **Invisorb**[®] **Blood Mini HTS 96 Kit /ep**. The procedure consistently delivered high molecular weight DNA as indicated by clear bands without detectable RNA contamination. The DNA was suitable for PCR amplification which is demonstrated by the successful amplification of the GAPDH - sequence in the samples (**Fig. 10**).

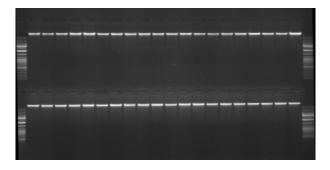


Fig. 9: Extracted genomic DNA from 40 isolated DNA samples were analyzed on 1% TAE agarose gel stained with ethidium bromide.

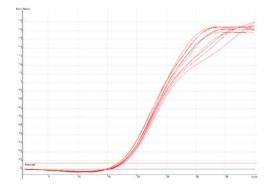


Fig. 10: Amplification of GAPDH out of 10 randomly taken samples

Selected reference:

Kretschmann, T. Reproducible, convenient walk-away purification of genomic DNA from whole blood with the Invisorb[®] Blood Mini HTS 96 Kit/ ep on the epMotion[®] 5075 VAC from Eppendorf; STRATEC Molecular Application Note AN 8C3aR/Eppendorf/09/2007

Genomic DNA isolation from large blood samples

To check if the automated process of genomic DNA isolation from large volumes of human blood ensures precise and reliable pipetting within each well without affecting adjacent wells a cross contamination test was performed. A chessboard pattern was prepared with alternating wells of water and separate prepared lymphocyte pellets from 2 ml whole blood. Genomic DNA was automatically extracted using the **Invisorb**[®] **Blood Midi HTS 96 Kit/ ep** and all eluted samples were subjected to an agarose gel electrophoresis analysis and a PCR as a sensitive method for detecting any contaminating DNA in the negative samples. The isolated genomic DNA is shown in **Fig. 11**. The results of the PCR amplification using the same samples are shown in **Fig. 12**. In the negative samples an amplification of the *mdm2* sequence was not detected.

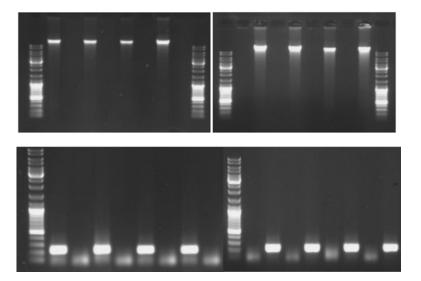


Fig. 11: 8 samples (3 buffy coat samples, and 5 different blood samples) and 8 no sample controls were arranged in alternating wells in a chessboard pattern. 10 μ l of the DNA eluates were analyzed on a 1% agarose gel.

Fig. 12: A mdm2-PCR was performed with an in-house method. 5 μ l of the PCR products were analyzed on a 1% agarose gel. The isolated DNA of buffy coat and blood samples is amplificable. There are no PCR products using the no sample controls. But an amplification of primer dimers is visible.

Genomic DNA isolation from SalivaGene[®] stabilized swabs

The **SalivaGene**[®] system combines the use of pre-filled SalivaGene[®] Collection Tubes for swab sample collection, the storage and stabilization of swab specimen without any degradation of the DNA during transportation. The SalivaGene[®] system further realizes the pre-lysis of bacteria and a very efficient and fast isolation of high-quality total DNA. The stabilized sample may be shipped in the SalivaGene[®] DNA Stabilizer containing tubes during 12 month at ambient temperature.

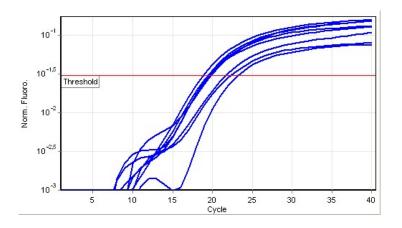


Fig. 13: Genomic DNA was isolated using the PSP[®] SalivaGene DNA HTS 96 Kit/ ep from SalivaGene[®] stabilized swabs from different donors stored at room temperature for six weeks. The GAPDH sequence was amplified in a real-time PCR.

1. Bacterial DNA extraction from a dilution series of Bacillus subtilis

To show the reproducibility and the sensitivity of the bacterial DNA isolation using the **Invisorb**[®] **Universal Bacteria HTS 96 Kit/ ep** different DNA extractions were performed from a dilution series. The real-time PCR results are shown in **Fig. 14**.

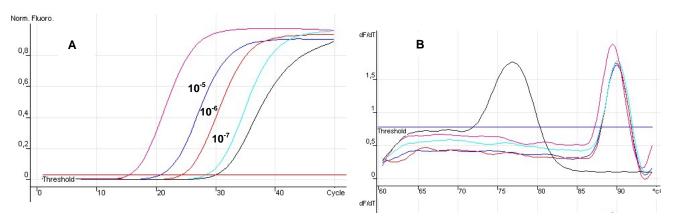


Fig. 14: Real-time PCR results using DNA extracted from a dilution series of B. subtilis cells in TE buffer (A). The melting curve for the resulting PCR products is shown (B). No template control (black) and positive template control (pink) are also shown.

2. Investigation of the influence of different matrices on the recovery of the bacterial DNA

To show the independence of the DNA isolation procedure from different pathogens containing matrices like plasma, TE buffer, swabs, blood or urine different kind of samples were spiked with the same amount of *B. subtilis* cells and DNA was isolated using the **Invisorb**[®] **Universal Bacteria HTS 96 Kit/ ep.** The recovery of the *B. subtilis* DNA from different matrices is nearly identical (**Fig. 15**).

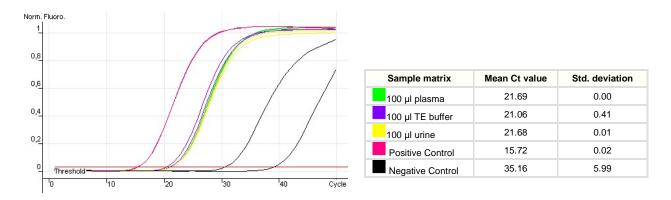


Fig. 15: Plasma (green), TE buffer (blue), and urine (yellow) were used. Samples were spiked with the same amount of B. subtilis cells. The negative control (black) and positive control (pink) are also shown. (Please see "Note" above).

Note: The late slope in the curves of NTC and cross contamination control reactions is not due to a specific PCR amplification. These signals come from unspecific products like primer dimers stained by the used SYBR Green reactions (for Bacillus subtilis and GAPDH). All products were analyzed by a melting curve and all were free of specific amplificates.

Fully automated viral DNA isolation

1. PCR inhibitor and cross contamination test

To maximize the detection of any potential contamination event, positive and no template controls were arranged in alternating wells. **Fig. 16** shows a real-time PCR run of the extracted viral DNA. PCR reactions were done with a Primer/Probe HBV set on a Rotor Gene[™] 3000.

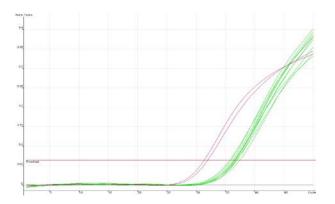
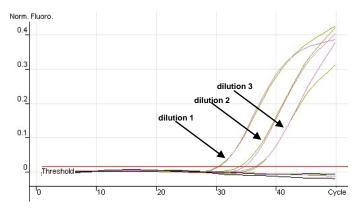


Fig.16: Real-time PCR results from 12 positive plasma samples (green), 12 negative samples (grey) and positive control (pink) are shown.

2. Reproducible and sensitive isolation of viral DNA from HBV spiked samples

Different viral DNA extractions were performed from a dilution series of CMV spiked plasma using the **Invisorb**[®] Virus DNA HTS 96 Kit/ ep. From a 1:10.000 dilution four further dilutions of 1:10 ($10^{-5} - 10^{-8}$) were made and extracted from plasma samples. The figure shows the amplification results for 3 different concentrations of CMV in plasma. The corresponding Ct values and standard deviations for the real-time PCR are listed below.



Sample	Mean Ct value	Std. deviation
CMV plasma dilution 1	30.75	0.11
CMV plasma dilution 2	34.63	0.56
CMV plasma dilution 3	37.02	0.20
CMV plasma dilution 1	30.83	
CMV plasma dilution 2	35.03	
CMV plasma dilution 3	36.88	

Fig. 17: Real-time PCR amplification curves from a CMV dilution series in plasma

1. Sensitive detection of viral RNA in cerebrospinal fluid from different patients

Cerebrospinal fluid samples from different encephalitis patients were used to detect human Enteroviruses. Viral RNA was isolated using the **Invisorb**[®] Virus RNA HTS 96 Kit/ ep. The "ENTEROVIRUS Q-PCR Alert AmpliMIX" real-time PCR assay (Nanogen Advanced Diagnostics) was used to detect and dose human Enterovirus cDNA belonging to serotypes: Poliovirus 1-3, Coxsackievirus A1-A22 and A24, Coxsackievirus B1-B6, Echovirus 1-9, 11-21, 24-27 and 29-33, Enterovirus 68-71. The assay cannot detect and dose human Parechovirus cDNA, previously known as Echovirus 22 and 23.

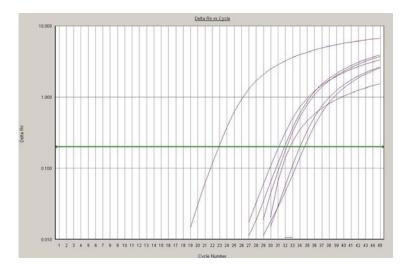


Fig. 18: Real-time PCR amplification curves from Enteroviruses isolated from CSF

2. Virus RNA extraction from different starting materials

A test using urine, rinse fluid from cervix swabs from different patients and plasma as control was performed using the **Invisorb[®] Virus RNA HTS 96 Kit/ ep**. All samples were spiked with the same lot of Influenza A virus with a dilution of 1:200. The figure shows that the extraction efficiency is independent from the matrix.

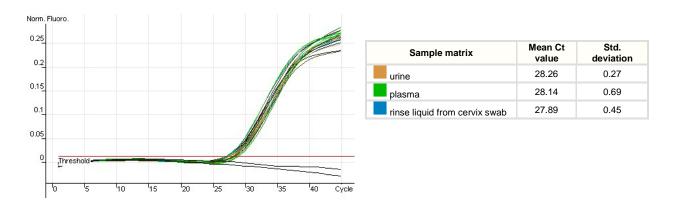


Fig. 19: Real-time PCR amplification curves from Influenza A in different matrices

Ordering information and run file names

Product name	Article number	Package size	Run file names
MSB [®] HTS PCRapace/ ep	7121240200 7121240300 7121240400	2 x 96 preps 4 x 96 preps 24 x 96 preps	Vac Version: PCRcleanupW TMX Version: PCRcleanupT
Invisorb [®] Plasmid HTS 96 Kit/ ep	7110320200 7110320300 7110320400	2 x 96 preps 4 x 96 preps 24 x 96 preps	Vac Version: InvPlm TMX Version: InvPlmTM
Invisorb [®] Blood Mini HTS 96 Kit/ ep	7131320200 7131320300 7131320400	2 x 96 preps 4 x 96 preps 24 x 96 preps	Vac Version: Blood mini W TMX Version: Blood mini_TMX
Invisorb [®] Blood Midi HTS 96 Kit/ ep	7131720200 7131720300 7131720400	2 x 96 preps 4 x 96 preps 24 x 96 preps	Blood Midi W.ws
PSP [®] SalivaGene DNA HTS 96 Kit/ ep	7136260200 7136260300 7136260400	2 x 96 preps 4 x 96 preps 24 x 96 preps	on request
Invisorb [®] Universal Bacteria HTS 96 Kit/ ep	7138320200 7138320300 7138320400	2 x 96 preps 4 x 96 preps 24 x 96 preps	Vac Version: UniversalBAC_W TMX Version: UniversalBAC_TMX
Invisorb [®] Virus RNA HTS 96 Kit/ ep	7143320200 7143320300 7143320400	2 x 96 preps 4 x 96 preps 24 x 96 preps	Vac Version: Virus RNA W TMX Version: Virus RNA_TMX
Invisorb [®] Virus DNA HTS 96 Kit/ ep	7142320200 7142320300 7142320400	2 x 96 preps 4 x 96 preps 24 x 96 preps	Vac Version: Virus DNA W TMX Version: Virus DNA_TMX

Ordering information - epMotion®

Eppendorf epMotio	on [®] processing products	
Cat-No.	Product	Supplier
5075 000.016	epMotion 5075 VAC, incl. control panel, software, optic sensor, disposables, MMC and card reader, manual 50/60Hz, 230 V (with integrated vacuum-station)	Eppendorf
5075 000.008	epMotion 5075 LH, 230 V; liquid handling station	Eppendorf
Conversion kit		
5075 000.610	VAC for conversion of a epMotion 5075 LH into a VAC- version	Eppendorf
Dosage instruments		
5280 000.010	TS 50, One-channel-dosage-instrument for 1 - 50 μ l	Eppendorf
5280 000.037	TS 300, One-channel-dosage-instrument for 20 - 300 μl	Eppendorf
5280 000.053	TS 1000, One-channel-dosage-instrument for 40 -1000 μΙ	Eppendorf
5280 000.215	TM 50-8, 8-channel-dosage-instrument for 1 - 50 µl	Eppendorf
5280 000.231	TM 300-8, 8-channel-dosage-instrument for 20 - 300 µl	Eppendorf
5280 000.258	TM 1000-8, 8-channel-dosage-instrument for 40 - 1.000 μΙ	Eppendorf
5075 774.003	Bracket for up to 6 dosage-instruments	Eppendorf
Consumables for e	epMotion [®] 5075 VAC	
0030 003.942	50 μl, volume range 1 - 50 μl, 15 x 96 tips in racks, Eppendorf quality	Eppendorf
0030 003.950	50 μl, filter tips, volume range 1 - 50 μl, 15 x 96 filter tips in racks, PCR-clean	Eppendorf
0030 003.969	300 μl, volume range 20 - 300 μl, 15 x 96 tips in tacks, Eppendorf quality	Eppendorf
0030 003.977	300 μl, filter tips, volume range 20 - 300 μl, 15 x 96 filter tips in racks, PCR-clean	Eppendorf
0030 003.985	1.000 μl, volume range 40 - 1.000 μl, 15 x 96 tips in racks, Eppendorf quality	Eppendorf
0030 003.993	1.000 μl, filter tips, volume range 40 - 1.000 μl, 15 x 96 filter tips in racks, PCR-clean	Eppendorf
5075 754.002	Reservoir rack	Eppendorf
0030 126.505	30 ml epMotion reservoir	Eppendorf
0030 126.513	100 ml epMotion reservoir	Eppendorf
5075 751.054	Thermo adapter for deep well plates	Eppendorf

About STRATEC Molecular

STRATEC Molecular – part of the STRATEC group since 2009 – is a globally active provider of innovative system solutions for nucleic acid sample collection, stabilization, and both manual and automated purification from any sample type. Since 1992 the company is internationally respected for its outstanding and high performance technology platforms and offers a broad spectrum of superior products for molecular diagnostics, drug discovery and Life Science research.

As an EN ISO 13485:2003 + AC 2007 and EN ISO 9001:2008 certified company all STRATEC Molecular products are subject to extensive quality control. In compliance with Directive 98/79/EC (IVDD) many STRATEC Molecular products are CE-certified. STRATEC Molecular guarantees the correct function of all products and highest quality support by first-rate service.

About the STRATEC group

The STRATEC group consists of the publicly listed parent company STRATEC Biomedical AG and of subsidiaries and second-tier subsidiaries in Germany, the USA, the UK, Switzerland and Romania. The STRATEC Biomedical AG (<u>http://www.stratec.com</u>) designs and manufactures fully automated systems for its partners in the fields of clinical diagnostics and biotechnology.



Core technologies

Non-chaotropic chemistry

- shorter protocols through reduced salt concentrations
- higher yields from complex/precious samples
- more intact chromosomal DNA

MSB[®] - Minimal salt binding

- the fastest way to purify DNA fragments
- excellent purity without washing

RTP[®] - Ready to prep

- Extraction Tube provides all lysis components, Carrier RNA and standards stabilized at RT
- safer sample handling due to reduced hands-on steps

PSP[®] - DNA sample stabilization

- stabilization of host/pathogen DNA at RT in stool, saliva or swab samples
- preservation of bacterial titers at the time of sampling

InviTrap[®] - Selective removal of DNA

- highly purified RNA for better RT-PCR results
- no DNase digestion required

InviMag[®] - Magnetic beads

- manual and automated DNA and RNA purification

RNAsure[®] - Viral RNA protection

- immediate lysis and viral RNA stabilization in biological samples
- RNA resistant to degradation for up to 6 month at RT