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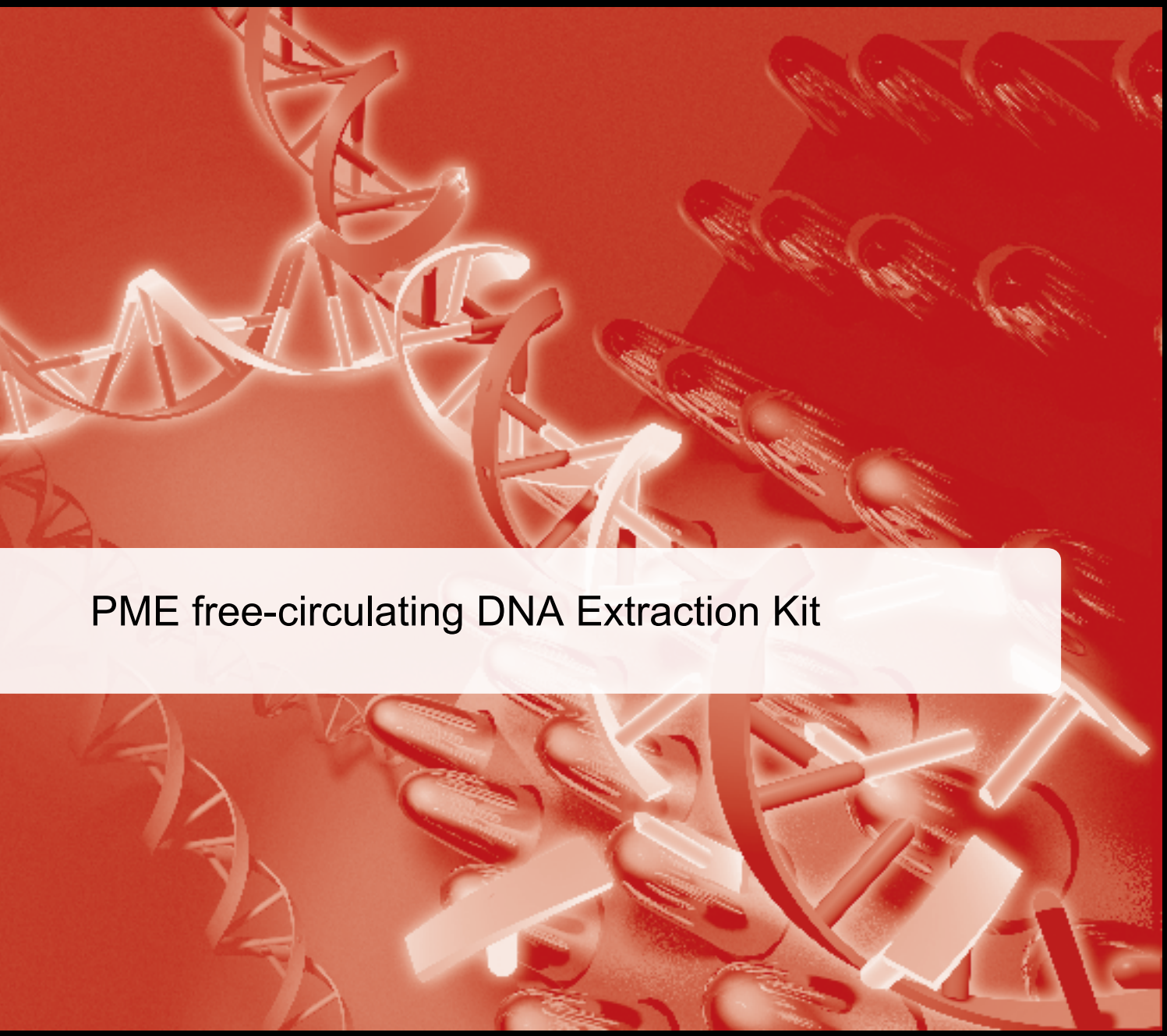
Manual



PME free-circulating DNA Extraction Kit

Life Science unlimited

Order No.:

845-IR-0003010	10 reactions
845-IR-0003050	50 reactions

Publication No.: HB_IR-0003_e_160620

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AJ Innuscreen GmbH
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Made in Germany!

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Contents

1 Introduction

1.1 Intended use

Free-circulating DNA in serum, plasma or in urine is very interesting as diagnostic target. The content of free-circulating DNA is usually very low and varies among different individuals. Further, the free-circulating DNA is present as short fragments, usually smaller than 1000 nt. Because of these facts, the extraction of cell-free-circulating DNA is difficult. Commercially available kits use standard nucleic acid extraction procedures based on sample lysis, binding the nucleic acids on a solid material, washing and elution of nucleic acids. Because of the high sample volume the procedures are very time and work consuming and need a lot of reagents.

The PME free-circulating DNA Extraction Kit is based on new technology, called: PME – Polymer Mediated Enrichment. The procedure doesn't start with sample lysis, like common used methods or kits. The first step is capturing of free-circulating DNA with a special polymer. Subsequently the captured free-circulating DNA is dissolved in a special buffer and then the DNA is extracted. The whole procedure for the isolation of free-circulating DNA from 1 ml of sample volume needs approx. 30 minutes and from 2 ml to 5 ml serum or plasma or 5 ml to 10 ml urine less than 1 hour.

The PME free-circulating DNA Extraction Kit contains two different Lysis/Binding Solution systems. Both systems are applicable for extraction of free-circulating DNA. Further, the kit contains a Carrier RNA. Addition of Carrier RNA is recommended if extreme low amount of free-circulating DNA is expected. In this case the addition of Carrier RNA can increase the final yield. Using Real-time PCR as a downstream application has shown a benefit of 0.5 – 1 Ct-value. In all other cases the addition of Carrier RNA is not necessary.

The kit works with 1 ml to 5 ml serum or plasma or 5 ml to 10 ml urine sample. The extracted free-circulating DNA is suitable for downstream applications like PCR, Real-time PCR, bisulfite conversion or any kind of enzymatic reaction.

The detection limit for certain free-circulating DNA depends on the individual procedures, for example in-house PCR or commercial used detection assays.

Please note that in case of using the Carrier RNA the eluates contain free-circulating DNA and Carrier RNA. In case the extracted nucleic acids are not suitable for some downstream applications like next generating sequencing (NGS) or the quantification of nucleic acids (isolated with this kit) by photometric or fluorometric methods. It is recommended to quantify extracted DNA with other methods like specific quantitative PCR or Real-time PCR, or not to use the Carrier RNA. Furthermore, Carrier RNA may inhibit PCR reactions. Thus the amount of add Carrier RNA has to be carefully optimized depending on the individual PCR system used.

Consult instruction for use

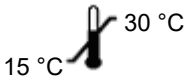
This package insert must be read carefully prior to use. Package insert instructions must be followed accordingly. Reliability of results cannot be guaranteed if there are any deviations from the instructions in this package insert. Prior using short manuals, first the detailed manuals are to be used.

1.2 Notes on the use of this manual

For easy reference and orientation, the manual uses the following warning and information symbols as well as the shown methodology:

REF
Catalogue number

Content
Contains sufficient reagents for <N> reactions



Storage conditions
Store at room temperature or shown conditions respectively

Consult instructions for use
This information must be observed to avoid improper use of the kit and the kit components.

Used by
Expiry date.

Lot number
The number of the kit charge

Manufactured by
Contact information of manufacturer

For single use only
Do not use components for a second time.

Note / Attention
Observe the notes marked in this way to ensure correct function of the device and to avoid operating errors for obtaining correct results.

The following systematic approach is introduced in the manual:

- The chapters and figures are numbered consecutively.
- A cross reference is indicated with an arrow (e.g. → "Notes on the use of this manual" p. 4).
- Working steps are numbered.

2 Safety precautions

Note

Read through this chapter carefully prior to guarantee your own safety and a trouble-free operation.

Follow all the safety instructions explained in the manual, as well as all messages and information, which are shown.

All due care and attention should be exercised in handling the materials and reagents contained in the kit. Always wear gloves while handling these reagents and avoid any skin contact! In case of contact, flush eyes or skin with a large amount of water immediately.

For single use only!

This kit is made for single use only!

Attention!

Don’t eat or drink components of the kit!

The kit shall only be handled by educated personal in a laboratory environment!

If the buffer bottles are damaged or leaking, wear gloves and protective goggles when discarding the bottles in order to avoid any injuries. Analytik Jena AG has not tested the liquid waste generated during using the kit for potential residual infectious components. This case is highly unlikely, but cannot be excluded completely. Therefore, all liquid waste must be considered as potentially infectious and must be handled and discarded according to local safety regulation.

Clinical sample must always be considered as potentially infectious. Samples from risk patients must always be labeled and handled under consequent safety conditions. Please observe the federal, state and local safety and environmental regulations. Observe the usual precautions for applications using extracted nucleic acids. All materials and reagents used for DNA or RNA isolation should be free of DNases or RNases.

Attention!

Do not add bleach or acidic components to the waste after sample preparation!

Note

Emergency medical information in English and German can be obtained 24 hours a day from:

Poison Information Center, Freiburg / Germany

Phone: +49 (0)761 19 240.

For more information, please ask for the material safety data sheet (MSDS).

3 Storage conditions

The PME free-circulating DNA Extraction Kit should be stored dry, at room temperature (15–30 °C) and is stable for at least 12 months under these conditions. If there are any precipitates within the provided solutions solve these precipitates by careful warming. For further information see table kit components (→ "Kit components" p. 7).

4 Function testing and technical assistance

The Analytik Jena AG guarantees the correct function of the kit for applications as described in the manual. The components of each PME free-circulating DNA Extraction Kit were tested by isolation of free-circulating DNA from human plasma/serum sample and analyzed in Real-time PCR.

We reserve the right to change or modify our products to enhance their performance and design. If you have any questions or problems regarding any aspects of the PME free-circulating DNA Extraction Kit or other Analytik Jena AG products, please do not hesitate to contact us. For technical support or further information in Germany please dial +49 36 41 / 77 94 00. For other countries please contact your local distributor.

5 Product use and warranty

The user is responsible to validate the performance of the Analytik Jena AG kits for any particular use, since the performance characteristics of our kits have not been validated for any specific application. Analytik Jena AG kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA' 88 regulations in the U.S. or equivalents in other countries.

All products sold by the Analytik Jena AG are subjected to extensive quality control procedures and are warranted to perform as described when used correctly. Any problems should be reported immediately.

Note

For research use only!

6 Kit components

Important

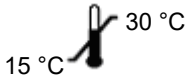
Store Enrichment Reagent VCR-1 at 4–8 °C!

Store lyophilized Proteinase K at 4–8 °C!

Divide dissolved Proteinase K into aliquots and storage at -22 to -18 °C is recommended. Repeated freezing and thawing will reduce the activity dramatically!

Store lyophilized Carrier RNA at -22 to -18 °C!

It is recommended to divide dissolved Carrier RNA stock solution into aliquots for storage at -22 to -18 °C. Do not freeze and thaw Carrier RNA stock solution more than 3 times.



Storage conditions

All other components are stored at room temperature.

	Σ 10	Σ 50
REF	845-IR-0003010	845-IR-0003050
Enrichment Reagent VCR-1	1.2 ml	5 x 1.2 ml
Enrichment Reagent VCR-2	10 ml	32 ml
Lysis Solution GS	8 ml	32 ml
Lysis Solution SE	6 ml	25 ml
Lysis Solution SEP	8 ml	32 ml
Carrier RNA	1x lyophilized powder	1x lyophilized powder
Proteinase K	for 2 x 0.3 ml working solution	for 2 x 1.5 ml working solution
Binding Solution VL	6 ml	20 ml
Binding Solution SBS	8 ml	32 ml
Washing Solution GS	15 ml (ready to use)	2 x 40 ml (ready to use)
Washing Solution BS	1 ml (final volume 10 ml)	5 ml (final volume 50 ml)
RNase-free Water	2.0 ml	2 x 2.0 ml
Spin Filter	10	50
Receiver Tubes (2.0 ml)	60	6 x 50
Elution Tubes (1.5 ml)	10	50
Manual	1	1

	Σ 10	Σ 50
REF	845-IR-0003010	845-IR-0003050
Initial steps	• Add 9 ml of 96-99.8 % ethanol to the bottle Washing Solution BS, mix thoroughly and keep the bottle always firmly closed! • Dissolve Proteinase K by addition of 0.3 ml of ddH_2O, mix thoroughly and store as described above! • Add 1 ml RNase-free Water to each tube Carrier RNA, mix thoroughly by pipetting up and down and store as described above!	• Add 45 ml of 96-99.8 % ethanol to the bottle Washing Solution BS, mix thoroughly and keep the bottle always firmly closed! • Dissolve Proteinase K by addition of 1.5 ml of ddH_2O, mix thoroughly and store as described above! • Add 1 ml RNase-free Water to each tube Carrier RNA, mix thoroughly by pipetting up and down and store as described above!

7 Recommended steps before starting

- Heat thermal mixer or water bath at 70 °C
- Pre-heat RNase-free Water at 70 °C.

Note: Do not use pre-heat RNase-free Water for Carrier RNA.

- Ensure that the Washing Solution BS, Proteinase K and Carrier RNA have been prepared according to the instruction (→ "Kit components" p. 7).
- Centrifugation steps should be carried out at room temperature
- Avoid freezing and thawing of starting material

8 Components not included in the kit

- 1.5 ml reaction tubes
- 15 ml reaction tubes
- 96–99.8 % ethanol (absolute, no denatured ethanol)
- ddH_2O for dissolving Proteinase K

9 GHS classification

Component	Hazard contents	GHS Symbol	Hazard phrases	Precaution phrases
Lysis Solution SE	Guanidinium chloride 10–25 % Edetic acid 2.5–<10 % Polyethylene glycol octylphenol ether 2.5–<10 %	 *Danger*	315, 318 412	101, 102, 103, 280,305+351+338, 362, 302+352, 403, 501
Lysis Solution SEP	Guanidinium chloride 10–25 % Edetic acid 2.5–<10 % Polyethylene glycol octylphenol ether 2.5–<10 %	 *Danger*	315, 318 412	101, 102, 103, 280,305+351+338, 362, 302+352, 403, 501
Enrichment Reagent VCR-2	Calcium chloride dihydrate	 *Warning*	319	101, 102, 103, 280,305+351+338,
Lysis Solution GS	Guanidinium thiocyanate 50–100 % Acetic acid 1–<2.5 % Polyethylene glycol octylphenol ether 0.3–<1.0 %	 *Danger*	302+312 +332, 314, 412	101, 102, 103, 260,303+361+353, 305+351+338, 310, 405, 501
Binding Solution SBS	Propan-2-ol 50–100 %	 *Danger*	225, 319, 336	101, 102, 103, 210, 261, 303+361+353, 305+351+338, 405, 501
Proteinase K	Proteinase, Tritirachium album serine	 *Danger*	315, 317, 319, 334, , 335	101, 102, 103, 261, 280, 305+351+338, 342+311, 405, 501
Washing Solution GS	Guanidinium thiocyanate 50–100 % Acetic acid 1–<2.5 % Polyethylene glycol octylphenol ether 0.3–<1.0 %	 *Danger*	302+312 +332, 314, 412	101, 102, 103, 260,303+361+353, 305+351+338, 310, 405, 501

Caution: DO NOT add bleach or acidic solutions directly to the sample-preparation waste.

Hazard phrases

225 Highly flammable liquid and vapor.

302 Harmful if swallowed.

314 Causes severe skin burns and eye damage.

315 Causes skin irritation.

317 May cause an allergic skin reaction.

318 Causes serious eye damage.

319 Causes serious eye irritation.

334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.

335 May cause respiratory irritation.

336 May cause drowsiness or dizziness.

411 Toxic to aquatic life with long lasting effects.

412 Harmful to aquatic life with long lasting effects.

302+312+332 Harmful if swallowed, in contact with skin if inhaled.

Precaution phrases

101 If medical advice is needed, have product container or label at hand.

102 Keep out of reach of children.

103 Read label before use.

210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.

260 Do not breathe dust/fume/gas/mist/vapors/spray.

261 Avoid breathing dust/fume/gas/mist/vapors/spray.

280 Wear protective gloves/protective clothing/ eye protection/face protection.

310 Immediately call a POISON CENTER/doctor.

362 Take off contaminated clothing.

403 Store in a well-ventilated place.

405 Store locked up.

501 Dispose of contents/container in accordance with local/regional/national/international regulations.

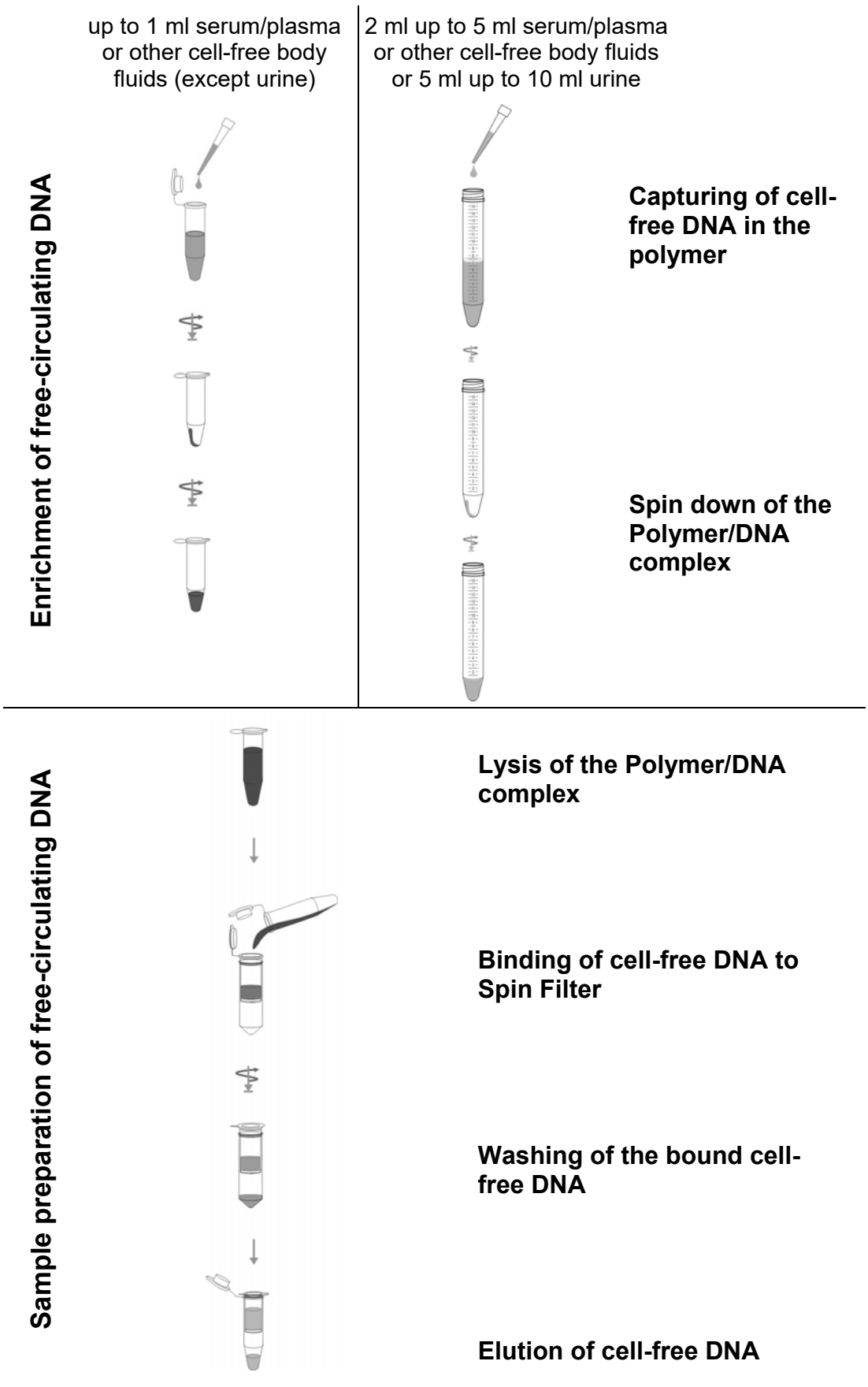
302+352 IF ON SKIN: Wash with plenty of water.

342+311 If experiencing respiratory symptoms: Call a POISON CENTER/doctor.

303+361+353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.

305+351+338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

10 General procedure of enrichment and isolation of free-circulating DNA



11 Product specifications

1. Starting material:

- Serum, plasma, cell culture supernatants or mediums and other cell-free body fluids from 1 ml up to 5 ml
- Urine from 5 ml to 10 ml

2. Time for isolation:

Approximately 30–60 minutes

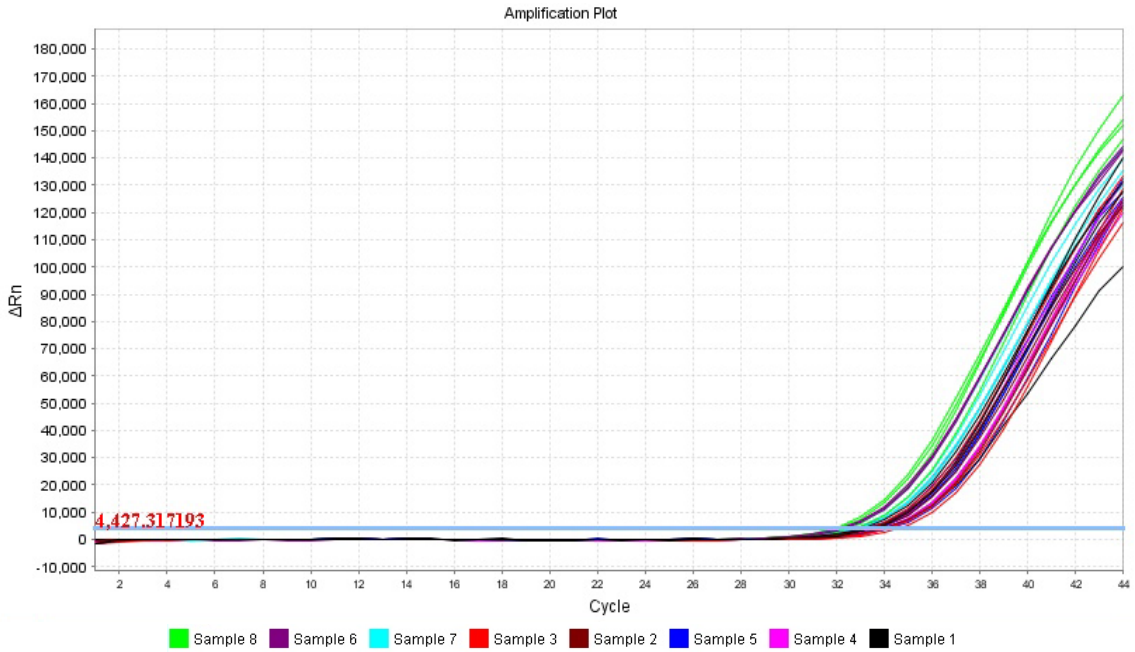
12 Validation results

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

Besides of the very different amount of free-circulating DNA from different individuals, also the blood collection systems have influence on the recovery of free-circulating DNA. At the moment we have tested the following blood collection systems:

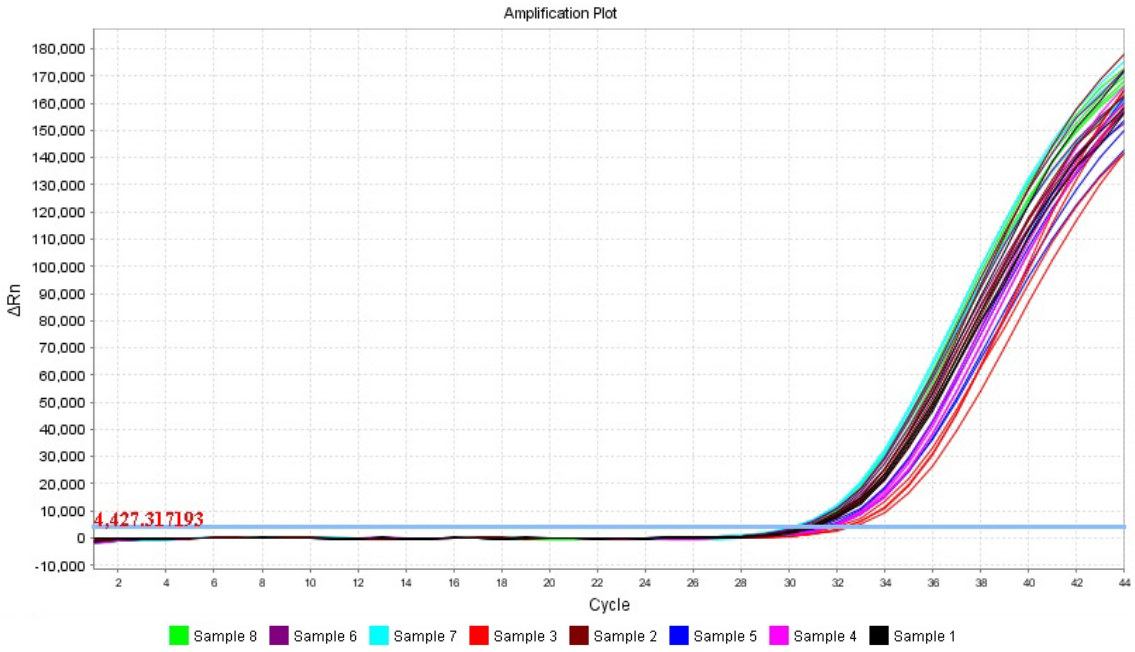
No.	Blood collecting system	Manufacturer
1	S-Monovette® 9 ml Silicat	Sarstedt
2	S-Monovette® 9 ml Polyacrylester Gel	Sarstedt
3	S-Monovette® 8.5 ml CPDA	Sarstedt
4	S-Monovette® 9 ml K3E (EDTA K3)	Sarstedt
5	S-Monovette® 10 ml 9NC (Trisodium Citrate Solution, Citrate Solution)	Sarstedt
6	S-Monovette® 7.5 ml NH (Natrium-Heparin)	Sarstedt
7	S-Monovette® 7.5 ml LH-Gel (Lithium-Heparin)	Sarstedt
8	S-Monovette® 9 ml LH (Lithium-Heparin)	Sarstedt

Extractions of free-circulating DNA from 1 ml of serum or plasma using the listed blood collection systems. Extracted free-circulating DNA has been tested by amplification of a human specific target gene:



▲ The amplification plots show differences depends on kind 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).

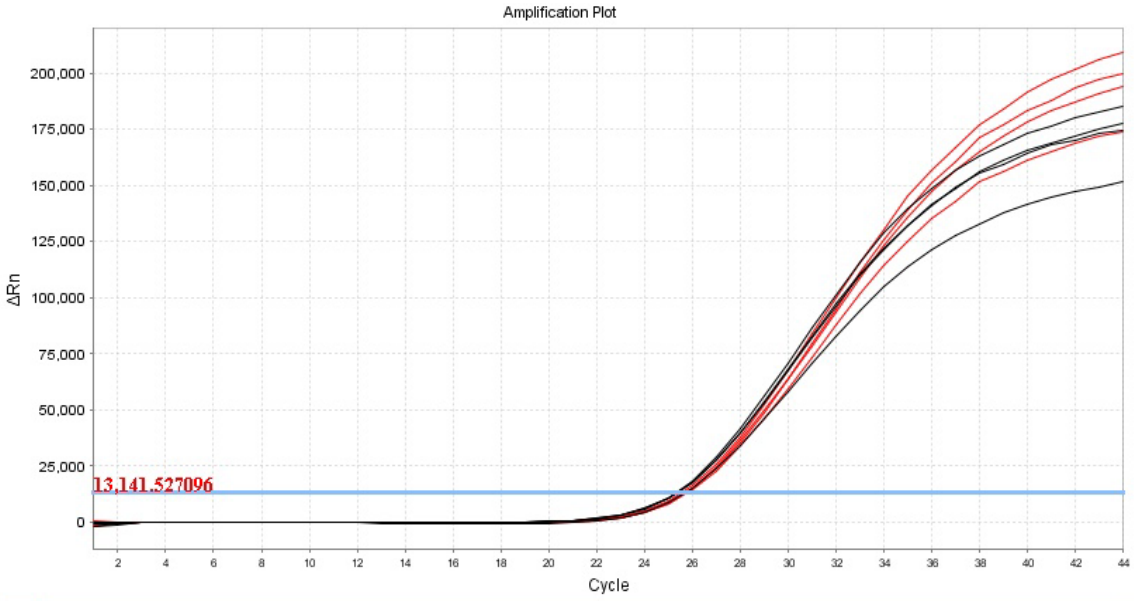
Extractions of free-circulating DNA from 5 ml of serum or plasma using the listed blood collection systems. Extracted free-circulating DNA has been tested by amplification of a human specific target gene:



▲ The amplification plots show differences depends on kind 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).

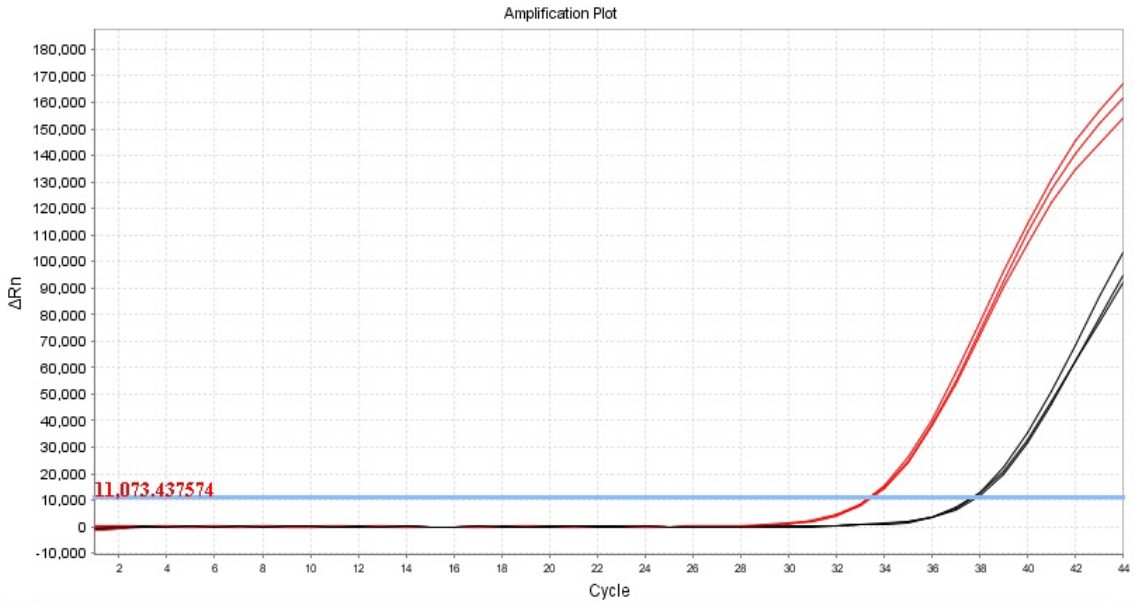
2. Comparison of Lysis Solution GS / Binding Solution VL and Lysis Solution SE / Binding Solution SBS system:

From 5 ml human serum the free-circulating DNA has been extracted by using Lysis Solution GS / Binding Solution VL and Lysis Solution SE / Binding Solution SBS system from PME free-circulating DNA Extraction Kit: The free-circulating DNA was compared by using human estrogen receptor 1 specific Real-time PCR.



▲ The black graphs correspond to the extraction of free-circulating DNA by using Lysis Solution GS / Binding Solution VL system and the red graphs to Lysis Solution SE / Binding Solution SBS system.

Extraction of free-circulating DNA from 5 ml human urine sample by using Lysis Solution GS / Binding Solution VL and Lysis Solution SE / Binding Solution SBS system. Following the extraction the free-circulating DNA was tested in Real-time PCR by amplifying a human specific coding gene.



▲ The black graphs correspond to the extraction of free-circulating DNA by using Lysis Solution GS / Binding Solution VL system and the red graphs to Lysis Solution SE / Binding Solution SBS system.

13 Selecting the optimal lysis solution system

The PME free-circulating DNA Extraction Kit contains two different Lysis/Binding Solution systems. Both systems are applicable for extraction of free-circulating DNA from different kind of starting materials.

Serum, plasma, cell culture supernatants or mediums and other cell-free body fluids differ from each other in their compositions. They also vary in the content and in the size of free-circulating DNA, e.g.: short DNA fragment or long DNA fragment. Therefore, all Lysis/Binding Solution systems are provided for optimal processing, purification performance, high yields and excellent quality of DNA.

In order to find optimal Lysis/Binding Solution systems when using a certain serum or plasma sample for the first time, it is recommended to do one by one preparations of one batch of sample with all Lysis/Binding Solution systems (see protocol 1 to 2 and 4 to 5) and make a results comparison (regarding yield of short or long fragment of DNA) to choose the best system for the sample.

For the urine sample, in order to get better results regarding DNA yield and removal of inhibitor in Real-time PCR, we recommend to use the Lysis Solution SEP / Binding Solution SBS system (protocol 3). Nevertheless, the Lysis Solution GS / Binding Solution VL system (protocol 6) could also be tested and used for individual urine sample.

14 Protocols using Lysis Solution SE / Binding Solution SBS system, Lysis Solution SEP / Binding Solution SBS system:

14.1 Protocol 1: Isolation of free-circulating DNA from serum, plasma, or other cell-free body fluids (except urine) up to 1 ml

Important
Pre-fill the needed amount of RNase-free Water in a 1.5 ml reaction tube and incubate the RNase-free Water at 70 °C until the elution step.

1. Add **30 µl of Enrichment Reagent VCR-1** and the sample into a 1.5 ml reaction tube and vortex shortly. Add **150 µl of Enrichment Reagent VCR-2** to the tube, mix shortly by vortexing. Incubate at room temperature for 1 minute.
2. Centrifuge at maximum speed for 3 minutes, open the tube and remove the supernatant carefully as much as possible.
3. Add 1 ml ddH_2O, invert the reaction tube three times and centrifuge at maximum speed for 3 minutes, open the tube and remove the supernatant carefully as much as possible.

   Note: Don’t remove the pellet; it will be processed like the following steps!
4. Add **400 µl Lysis Solution SE** to the reaction tube containing the pellet. Dissolve the pellet by pipetting up and down several times. Try to avoid thereby the formation of air bubbles!

   Note: Optional add 10 µl Carrier RNA to the sample after adding Lysis Solution SE. See → "Intended use" p. 3 if Carrier RNA is necessary to add or not!
5. Add **50 µl Proteinase K** to the reaction tube and mix vigorously by pulsed vortexing for 10 seconds. Incubate at 70 °C for 15 minutes under continuously shaking at 1,000 rpm.

   Note: We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3-4 times during incubation. No shaking will reduce the lysis efficiency. After lysis centrifuge the tube shortly to remove condensate from the lid of the tube.
6. Add **400 µl Binding Solution SBS** to the lysed sample, mix by pipetting up and down several times.

   Note: It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution and avoid the formation of air bubbles.

7. Apply **the whole sample** to the Spin Filter located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

8. Open the Spin Filter and add **500 µl Washing Solution GS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

9. Open the Spin Filter and add **650 µl Washing Solution GS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

10. Open the Spin Filter and add **650 µl Washing Solution BS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

11. Centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

12. Place the Spin Filter into a 1.5 ml Elution Tube. Open the cap of the Spin Filter and add **50 µl RNase-free Water** (pre-warmed at 70 °C). Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Two elution steps with equal volumes of RNase-free Water (e.g. 30 µl + 30 µl) might increase the yield of extracted free-circulating DNA.

Note

The free-circulating DNA can be eluted with a lower (minimum 30 µl) or a higher volume of RNase-free Water (depends on the expected yield of free-circulating DNA). Elution with lower volumes of RNase-free Water increases the final concentration of free-circulating DNA. Store the extracted free-circulating DNA at 4–8 °C. For long time storage placing at -22 to -18 °C is recommended.

14.2 Protocol 2: Isolation of free-circulating DNA from serum, plasma, or other cell-free body fluids (except urine) of 2 ml up to 5 ml

Important

Pre-fill the needed amount of RNase-free Water in a 1.5 ml reaction tube and incubate the RNase-free Water at 70 °C until the elution step.

1. Add **100 µl of Enrichment Reagent VCR-1** and the sample into a 15 ml reaction tube and vortex shortly. Add **600 µl of Enrichment Reagent VCR-2** to the tube, mix shortly by vortexing. Incubate at room temperature for 10 minutes.
2. Centrifuge the tubes at least at 4,200 x g (~5,000 rpm) for 10 minutes, open the tube and remove the supernatant carefully as much as possible.
3. Add 5 ml ddH_2O to the tube, invert the tube three times and centrifuge at least at 4,200 x g (~5,000 rpm) 5 minutes, open the tube and remove the supernatant carefully as much as possible.

   Note: Don’t remove the pellet; it will be processed like the following steps!
4. Add **600 µl Lysis Solution SEP** to the 15 ml reaction tube containing the pellet. Dissolve the pellet by pipetting up and down several times and transfer the suspension into a 1.5 ml reaction tube. Try to avoid thereby the formation of air bubbles!

   Note: Optional add 10 µl Carrier RNA to the sample after adding Lysis Solution SE. See → "Intended use" p. 3 if Carrier RNA is necessary to add or not!
5. Add **50 µl Proteinase K** to the reaction tube and mix vigorously by pulsed vortexing for 10 seconds. Incubate at 70 °C for 15 minutes under continuously shaking at 1,000 rpm.

   Note: We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3-4 times during incubation. No shaking will reduce the lysis efficiency. After lysis centrifuge the tube shortly to remove condensate from the lid of the tube.
6. Centrifuge at maximum speed for 2 minutes, open the tube and **transfer the supernatant** carefully in a new reaction tube.

   Note: If there is a pellet, don’t destroy it and pipette as much as possible from supernatant!
7. Add **600 µl Binding Solution SBS** to the lysate, mix by pipetting up and down several times.

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PME free-circulating DNA Extraction Kit (using SE/SBS system)

Protocol 1: Isolation of free-circulating DNA up to 1 ml

Recommended steps before starting

- Pre-heat the needed amount of RNase-free Water (70 °C)
- Prepare Washing Solution BS, Proteinase K and Carrier RNA according to the instruction
- Prepare shaking platform or water bath at 70 °C

1.	Starting material	Serum, Plasma, Cell-free body fluids (except urine)	▪ Add up to 1.0 ml to 1.5 ml reaction tube
2.	Capturing of cell-free DNA in the polymer		▪ Add 30 µl VCR-1 ▪ Vortex: 10 sec ▪ Add 150 µl VCR- 2 ▪ Vortex: 10 sec ▪ Incubation: 1 min @ RT
3.	Spin Down of Polymer/DNA complex	 	▪ Centrifuge: max speed, 3 min ▪ Open tube ▪ Remove and discard supernatant
4.	Washing of the Polymer/DNA complex	 	▪ Add 1 ml ddH_2O, invert the tube 3x **Note:** Don't remove the pellet! ▪ Centrifuge: max speed, 3 min ▪ Open the tube ▪ Remove and discard supernatant
5.	Lysis of the Polymer/DNA complex	 	▪ Add 400 µl Lysis Solution SE ▪ Dissolve pellet by pipetting up & down **Note:** Try to avoid air bubbles! **Optional:** Add Carrier RNA. ▪ Add 50 µl PK and vortex:10 sec ▪ Incubation: 15 min @ 70 °C, shaking : 1,000 rpm ▪ Centrifuge: shortly

✂ cut at the scattered line and laminate the card for a more convenient handling on the table top ✂

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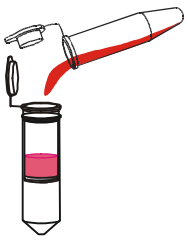
6. Binding of free-circulating DNA

New Receiver Tubes

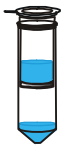


- Add 400 µl Binding Solution SBS
- Mix by pipetting up & down

  Note: Try to avoid air bubbles!
- Add Spin Filter to Receiver Tube
- Add sample to Spin Filter
- 11,000 x g (~12,000 rpm): 1 min

7. Washing

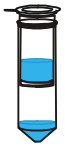
New Receiver Tubes



- Add 500 µl Washing Solution GS
- 11,000 x g (~12,000 rpm): 1 min
- Add 650 µl Washing Solution GS
- 11,000 x g (~12,000 rpm): 1 min
- Add 650 µl Washing Solution BS
- 11,000 x g (~12,000 rpm): 1 min

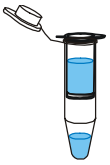
9. Remove Ethanol

New Receiver Tube



- Discard filtrate
- Add Spin Filter to Receiver Tube
- Centrifuge: max speed, 3 min

10. Elution



- Add Spin Filter to an Elution Tube
- Add 50 µl pre-heat RNase-free Water
- Incubation: 2 min @ RT
- 11,000 x g (~12,000 rpm): 1 min

Order No.:	845-IC-0003010	10 reactions
	845-IC-0003050	50 reactions

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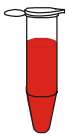
PME free-circulating DNA Extraction Kit(using SEP/SBS System)

Protocol 2: Isolation of free-circulating DNA of 2 ml up to 5 ml

Recommended steps before starting

- Pre-heat the needed amount of RNase-free Water (70 °C)
- Prepare Washing Solution BS, Proteinase K and Carrier RNA according to the instruction
- Prepare shaking platform or water bath at 70 °C

1.	Starting material	Serum, Plasma, Cell-free body fluids (except urine)	▪ Add 2.0 ml up to 5.0 ml to 15 ml reaction tube
2.	Capturing of cell-free DNA in the polymer		▪ Add 100 µl VCR-1 ▪ Vortex: 10 sec ▪ Add 600 µl VCR- 2 ▪ Vortex: 10 sec ▪ Incubation: 10 min @ RT
3.	Spin Down of Polymer/DNA complex	 	▪ 4,200 x g (~5,000 rpm): 10 min ▪ Open tube ▪ Remove and discard supernatant
4.	Washing of the Polymer/DNA complex	 	▪ Add 5 ml ddH_2O, invert the tube 3x **Note:** Don't remove the pellet! ▪ 4,200 x g (~5,000 rpm): 5 min ▪ Open the tube ▪ Remove and waste supernatant
5.	Lysis of the Polymer/DNA complex	 	▪ Add 600 µl Lysis Solution SEP ▪ Dissolve pellet by pipetting up & down **Note:** Try to avoid air bubbles! **Optional:** Add Carrier RNA. ▪ Transfer suspension to 1.5 ml tube
6.	Lysis of the Polymer/DNA complex	 	▪ Add 50 µl PK and vortex: 10 sec ▪ Incubation: 15 min @ 70 °C, shaking : 1,000 rpm ▪ Centrifuge: max. speed, 2 min ▪ Transfer supernatant to 1.5 ml tube

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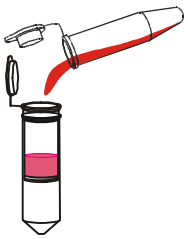
7. Binding of free-circulating DNA

New Receiver Tubes

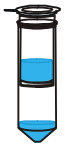


- Add 600 µl Binding Solution SBS
- Mix by pipetting up & down

  Note: Try to avoid air bubbles!
- Add Spin Filter to Receiver Tube
- Add 600 µl sample to Spin Filter
- 11,000 x g (~12,000 rpm): 1 min
- Add residual sample to Spin Filter
- 11,000 x g (~12,000 rpm): 1 min

8. Washing

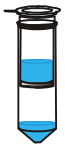
New Receiver Tubes



- Add 500 µl Washing Solution GS
- 11,000 x g (~12,000 rpm): 1 min
- Add 650 µl Washing Solution GS
- 11,000 x g (~12,000 rpm): 1 min
- Add 650 µl Washing Solution BS
- 11,000 x g (~12,000 rpm): 1 min

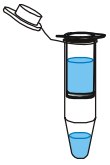
9. Remove Ethanol

New Receiver Tube



- Discard filtrate
- Add Spin Filter to Receiver Tube
- Centrifuge: max speed, 3 min

10. Elution



- Add Spin Filter to an Elution Tube
- Add 50 µl pre-heat RNase-free Water
- Incubation: 2 min @ RT
- 11,000 x g (~12,000 rpm): 1 min

Order No.:	845-IC-0003010	10 reactions
	845-IC-0003050	50 reactions

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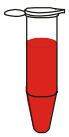
PME free-circulating DNA Extraction Kit(using SEP/SBS System)

Protocol 3: Isolation of free-circulating DNA of 5 ml up to 10 ml

Recommended steps before starting

- Pre-heat the needed amount of RNase-free Water (70 °C)
- Prepare Washing Solution BS, Proteinase K and Carrier RNA according to the instruction
- Prepare shaking platform or water bath at 70 °C

1.	Starting material	Urine sample	▪ Add 5.0 ml up to 10.0 ml to 15 ml reaction tube
2.	Capturing of cell-free DNA in the polymer		▪ Add 100 µl VCR-1 ▪ Vortex: 10 sec ▪ Add 600 µl VCR- 2 ▪ Vortex: 10 sec ▪ Incubation: 10 min @ RT
3.	Spin Down of Polymer/DNA complex	 	▪ 4,200 x g (~5,000 rpm): 10 min ▪ Open tube ▪ Remove and discard supernatant
4.	Washing of the Polymer/DNA complex	 	▪ Add 5 ml ddH_2O, invert the tube 3x **Note:** Don't remove the pellet! ▪ 4,200 x g (~5,000 rpm): 5 min ▪ Open the tube ▪ Remove and waste supernatant
5.	Lysis of the Polymer/DNA complex	 	▪ Add 600 µl Lysis Solution SEP ▪ Dissolve pellet by pipetting up & down **Note:** Try to avoid air bubbles! **Optional:** Add Carrier RNA. ▪ Transfer suspension to 1.5 ml tube
6.	Lysis of the Polymer/DNA complex	 	▪ Add 50 µl PK and vortex: 10 sec ▪ Incubation: 15 min @ 70 °C, shaking : 1,000 rpm ▪ Centrifuge: max. speed, 2 min ▪ Transfer supernatant to 1.5 ml tube

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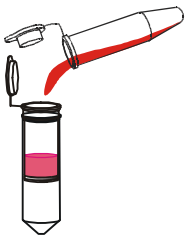
7. Binding of free-circulating DNA

New Receiver Tubes

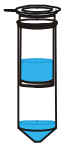


- Add 600 µl Binding Solution SBS
- Mix by pipetting up & down

  Note: Try to avoid air bubbles!
- Add Spin Filter to Receiver Tube
- Add 600 µl sample to Spin Filter
- 11,000 x g (~12,000 rpm): 1 min
- Add residual sample to Spin Filter
- 11,000 x g (~12,000 rpm): 1 min

8. Washing

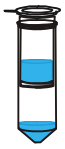
New Receiver Tubes



- Add 500 µl Washing Solution GS
- 11,000 x g (~12,000 rpm): 1 min
- Add 650 µl Washing Solution GS
- 11,000 x g (~12,000 rpm): 1 min
- Add 650 µl Washing Solution BS
- 11,000 x g (~12,000 rpm): 1 min

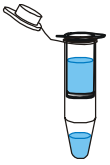
9. Remove Ethanol

New Receiver Tube



- Discard filtrate
- Add Spin Filter to Receiver Tube
- Centrifuge: max speed, 3 min

10. Elution



- Add Spin Filter to an Elution Tube
- Add 50 µl pre-heat RNase-free Water
- Incubation: 2 min @ RT
- 11,000 x g (~12,000 rpm): 1 min

Order No.:	845-IC-0003010	10 reactions
	845-IC-0003050	50 reactions

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PME free-circulating DNA Extraction Kit (using GS/VL system)

Protocol 4: Isolation of free-circulating DNA up to 1 ml

Recommended steps before starting

- Pre-heat the needed amount of RNase-free Water (70 °C)
- Prepare Washing Solution BS, Proteinase K and Carrier RNA according to the instruction
- Prepare shaking platform or water bath at 70 °C

1.	Starting material	Serum, Plasma, Cell-free body fluids (except urine)	▪ Add up to 1.0 ml to 1.5 ml reaction tube
2.	Capturing of cell-free DNA in the polymer		▪ Add 30 µl VCR-1 ▪ Vortex: 10 sec ▪ Add 150 µl VCR- 2 ▪ Vortex: 10 sec ▪ Incubation: 1 min @ RT
3.	Spin Down of Polymer/DNA complex	 	▪ Centrifuge: max speed, 3 min ▪ Open tube ▪ Remove and discard supernatant
4.	Washing of the Polymer/DNA complex	 	▪ Add 1 ml ddH_2O, invert the tube 3x **Note:** Don't remove the pellet! ▪ Centrifuge: max speed, 3 min ▪ Open the tube ▪ Remove and discard supernatant
5.	Lysis of the Polymer/DNA complex	 	▪ Add 400 µl Lysis Solution GS ▪ Dissolve pellet by pipetting up & down **Note:** Try to avoid air bubbles! **Optional:** Add Carrier RNA. ▪ Add 50 µl PK and vortex:10 sec ▪ Incubation: 15 min @ 70 °C, shaking : 1,000 rpm ▪ Centrifuge: shortly

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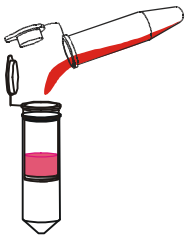
6. Binding of free-circulating DNA

New Receiver Tubes

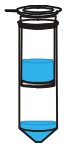


- Add 200 µl Binding Solution VL
- Mix by pipetting up & down

  Note: Try to Avoid air bubbles!
- Add Spin Filter to Receiver Tube
- Add sample to Spin Filter
- 11,000 x g (~12,000 rpm): 1 min

7. Washing

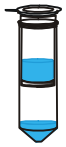
New Receiver Tubes



- Add 500 µl Washing Solution GS
- 11,000 x g (~12,000 rpm): 1 min
- Add 650 µl Washing Solution GS
- 11,000 x g (~12,000 rpm): 1 min
- Add 650 µl Washing Solution BS
- 11,000 x g (~12,000 rpm): 1 min

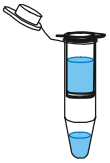
9. Remove Ethanol

New Receiver Tube



- Discard filtrate
- Add Spin Filter to Receiver Tube
- Centrifuge: max speed, 3 min

10. Elution



- Add Spin Filter to an Elution Tube
- Add 50 µl pre-heat RNase-free Water
- Incubation: 2 min @ RT
- 11,000 x g (~12,000 rpm): 1 min

Order No.:	845-IC-0003010	10 reactions
	845-IC-0003050	50 reactions

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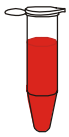
PME free-circulating DNA Extraction Kit (using GS/VL system)

Protocol 5: Isolation of free-circulating DNA of 2 ml up to 5 ml

Recommended steps before starting

- Pre-heat the needed amount of RNase-free Water (70 °C)
- Prepare Washing Solution BS, Proteinase K and Carrier RNA according to the instruction
- Prepare shaking platform or water bath at 70 °C

1.	Starting material	Serum, Plasma, Cell-free body fluids (except urine)	▪ Add 2.0 ml up to 5.0 ml to 15 ml reaction tube
2.	Capturing of cell-free DNA in the polymer		▪ Add 100 µl VCR-1 ▪ Vortex: 10 sec ▪ Add 600 µl VCR- 2 ▪ Vortex: 10 sec ▪ Incubation: 10 min @ RT
3.	Spin Down of Polymer/DNA complex	 	▪ 4,200 x g (~5,000 rpm): 10 min ▪ Open tube ▪ Remove and discard supernatant
4.	Washing of the Polymer/DNA complex	 	▪ Add 5 ml ddH_2O, invert the tube 3x **Note:** Don't remove the pellet! ▪ 4,200 x g (~5,000 rpm): 5 min ▪ Open the tube ▪ Remove and waste supernatant
5.	Lysis of the Polymer/DNA complex	 	▪ Add 600 µl Lysis Solution GS ▪ Dissolve pellet by pipetting up & down **Note:** Try to avoid air bubbles! **Optional:** Add Carrier RNA. ▪ Transfer suspension to 1.5 ml tube
6.	Lysis of the Polymer/DNA complex	 	▪ Add 50 µl PK and vortex: 10 sec ▪ Incubation: 15 min @ 70 °C, shaking : 1,000 rpm ▪ Centrifuge: max. speed, 2 min ▪ Transfer supernatant to 1.5 ml tube

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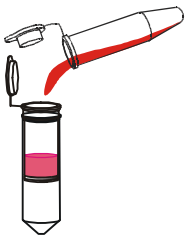
7. Binding of free-circulating DNA

New Receiver Tubes

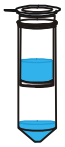


- Add 300 µl Binding Solution VL
- Mix by pipetting up & down

  Note: Try to avoid air bubbles!
- Add Spin Filter to Receiver Tube
- Add 600 µl sample to Spin Filter
- 11,000 x g (~12,000 rpm): 1 min
- Add residual sample to Spin Filter
- 11,000 x g (~12,000 rpm): 1 min

8. Washing

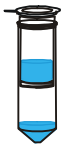
New Receiver Tubes



- Add 500 µl Washing Solution GS
- 11,000 x g (~12,000 rpm): 1 min
- Add 650 µl Washing Solution GS
- 11,000 x g (~12,000 rpm): 1 min
- Add 650 µl Washing Solution BS
- 11,000 x g (~12,000 rpm): 1 min

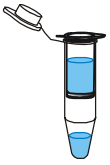
9. Remove Ethanol

New Receiver Tube



- Discard filtrate
- Add Spin Filter to Receiver Tube
- Centrifuge: max speed, 3 min

10. Elution



- Add Spin Filter to an Elution Tube
- Add 50 µl pre-heat RNase-free Water
- Incubation: 2 min @ RT
- 11,000 x g (~12,000 rpm): 1 min

Order No.:	845-IC-0003010	10 reactions
	845-IC-0003050	50 reactions

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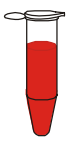
PME free-circulating DNA Extraction Kit (using GS/VL system)

Protocol 6: Isolation of free-circulating DNA of 5 ml up to 10 ml

Recommended steps before starting

- Pre-heat the needed amount of RNase-free Water (70 °C)
- Prepare Washing Solution BS, Proteinase K and Carrier RNA according to the instruction
- Prepare shaking platform or water bath at 70 °C

1.	Starting material	Urine sample	▪ Add 5.0 ml up to 10.0 ml to 15 ml reaction tube
2.	Capturing of cell-free DNA in the polymer		▪ Add 100 µl VCR-1 ▪ Vortex: 10 sec ▪ Add 600 µl VCR- 2 ▪ Vortex: 10 sec ▪ Incubation: 10 min @ RT
3.	Spin Down of Polymer/DNA complex	 	▪ 4,200 x g (~5,000 rpm): 10 min ▪ Open tube ▪ Remove and discard supernatant
4.	Washing of the Polymer/DNA complex	 	▪ Add 5 ml ddH_2O, invert the tube 3x **Note:** Don't remove the pellet! ▪ 4,200 x g (~5,000 rpm): 5 min ▪ Open the tube ▪ Remove and waste supernatant
5.	Lysis of the Polymer/DNA complex	 	▪ Add 600 µl Lysis Solution GS ▪ Dissolve pellet by pipetting up & down **Note:** Try to avoid air bubbles! **Optional:** Add Carrier RNA. ▪ Transfer suspension to 1.5 ml tube
6.	Lysis of the Polymer/DNA complex	 	▪ Add 50 µl PK and vortex: 10 sec ▪ Incubation: 15 min @ 70 °C, shaking : 1,000 rpm ▪ Centrifuge: max. speed, 2 min ▪ Transfer supernatant to 1.5 ml tube

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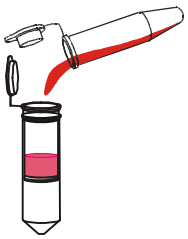
7. Binding of free-circulating DNA

New Receiver Tubes

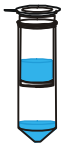


- Add 300 µl Binding Solution VL
- Mix by pipetting up & down

  Note: Try to avoid air bubbles!
- Add Spin Filter to Receiver Tube
- Add 600 µl sample to Spin Filter
- 11,000 x g (~12,000 rpm): 1 min
- Add residual sample to Spin Filter
- 11,000 x g (~12,000 rpm): 1 min

8. Washing

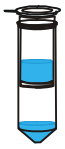
New Receiver Tubes



- Add 500 µl Washing Solution GS
- 11,000 x g (~12,000 rpm): 1 min
- Add 650 µl Washing Solution GS
- 11,000 x g (~12,000 rpm): 1 min
- Add 650 µl Washing Solution BS
- 11,000 x g (~12,000 rpm): 1 min

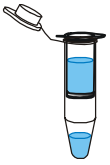
9. Remove Ethanol

New Receiver Tube



- Discard filtrate
- Add Spin Filter to Receiver Tube
- Centrifuge: max speed, 3 min

10. Elution



- Add Spin Filter to an Elution Tube
- Add 50 µl pre-heat RNase-free Water
- Incubation: 2 min @ RT
- 11,000 x g (~12,000 rpm): 1 min

Order No.:	845-IC-0003010	10 reactions
	845-IC-0003050	50 reactions

This documentation describes the state at the time of publishing. It needs not necessarily agree with future versions. Subject to change!

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lifescience@analytik-jena.com

Cut at the scattered line and laminate the card for a more convenient handling on the table top

Note: is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution and avoid the formation of air bubbles.

8. Apply **600 µl of the sample** to the Spin Filter located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

9. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube and load the residual sample onto the Spin Filter. Close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

10. Open the Spin Filter and add **500 µl Washing Solution GS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

11. Open the Spin Filter and add **650 µl Washing Solution GS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

12. Open the Spin Filter and add **650 µl Washing Solution BS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

13. Centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

14. Place the Spin Filter into a 1.5 ml Elution Tube. Open the cap of the Spin Filter and add **50 µl RNase-free Water** (prewarmed at 70 °C). Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Two elution steps with equal volumes of RNase-free Water (e.g. 30 µl + 30 µl) might increase the yield of extracted free-circulating DNA.

Note

The free-circulating DNA can be eluted with a lower (minimum 30 µl) or a higher volume of RNase-free Water (depends on the expected yield of

free-circulating DNA). Elution with lower volumes of RNase-free Water increases the final concentration of free-circulating DNA. Store the extracted free-circulating DNA at 4–8 °C. For long time storage placing at -22 to-18 °C is recommended.

14.3 Protocol 3: Isolation of free-circulating DNA from urine sample of 5 ml up to 10 ml

Important

Pre-fill the needed amount of RNase-free Water in a 1.5 ml reaction tube and incubate the RNase-free Water at 70 °C until the elution step.

Urine samples content cellular materials and cellular nucleic acids. In order to enrich only free-circulating DNA from the urine sample it is recommended to centrifuge the urine sample at maximum speed (e.g. 16,000 x g) and work subsequently only with the supernatant.

1. Add **100 µl of Enrichment Reagent VCR-1** and the sample into a 15 ml reaction tube and vortex shortly. Add **600 µl of Enrichment Reagent VCR-2** to the tube, mix shortly by vortexing. Incubate at room temperature for 10 minutes.
2. Centrifuge the tubes at least at 4,200 x g (~5,000 rpm) for 10 minutes, open the tube and remove the supernatant carefully as much as possible.
3. Add 5 ml ddH_2O to the tube, invert the tube three times and centrifuge at least at 4,200 x g (~5,000 rpm) 5 minutes, open the tube and remove the supernatant carefully as much as possible.

   Note: Don’t remove the pellet; it will be processed like the following steps!
4. Add **600 µl Lysis Solution SEP** to the 15 ml reaction tube containing the pellet. Dissolve the pellet by pipetting up and down several times and transfer the suspension into a 1.5 ml reaction tube. Try to avoid thereby the formation of air bubbles!

   Note: Optional add 10 µl Carrier RNA to the sample after adding Lysis Solution SE. See → "Intended use" p. 3 if Carrier RNA is necessary to add or not!
5. Add **50 µl Proteinase K** to the reaction tube and mix vigorously by pulsed vortexing for 10 seconds. Incubate at 70 °C for 15 minutes under continuously shaking at 1,000 rpm.

   Note: We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3-4 times during incubation. No shaking will reduce the lysis efficiency.
6. Centrifuge at maximum speed for 2 minutes, open the tube and **transfer the supernatant** carefully in a new reaction tube.

   Note: If there is a pellet, don’t destroy it and pipette as much as possible from supernatant!
7. Add **600 µl Binding Solution SBS** to the lysate, mix by pipetting up and down several times.

Note: It is important that the sample and the Binding Solution SBS are mixed vigorously to get a homogeneous solution and avoid the formation of air bubbles.

8. Apply **600 µl of the sample** to the Spin Filter located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

9. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube and load the residual sample onto the Spin Filter. Close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

10. Open the Spin Filter and add **500 µl Washing Solution GS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

11. Open the Spin Filter and add **650 µl Washing Solution GS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

12. Open the Spin Filter and add **650 µl Washing Solution BS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

13. Centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

14. Place the Spin Filter into a 1.5 ml Elution Tube. Carefully open the cap of the Spin Filter and add **50 µl RNase-free Water** (prewarmed at 70 °C). Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Two elution steps with equal volumes of RNase-free Water (e.g. 30 µl + 30 µl) might increase the yield of extracted free-circulating DNA.

Note

The free-circulating DNA can be eluted with a lower (minimum 30 µl) or a

higher volume of RNase-free Water (depends on the expected yield of free-circulating DNA). Elution with lower volumes of RNase-free Water increases the final concentration of free-circulating DNA. Store the extracted free-circulating DNA at 4–8 °C. For long time storage placing at -22 to -18 °C is recommended.

15 Protocols using Lysis Solution GS / Binding Solution VL system:

15.1 Protocol 4: Isolation of free-circulating DNA from serum, plasma, or other cell-free body fluids (except urine) up to 1 ml

Important

Pre-fill the needed amount of RNase-free Water in a 1.5 ml reaction tube and incubate the RNase-free Water at 70 °C until the elution step.

1. Add **30 µl of Enrichment Reagent VCR-1** and the sample into a 1.5 ml reaction tube and vortex shortly. Add **150 µl of Enrichment Reagent VCR-2** to the tube, mix shortly by vortexing. Incubate at room temperature for 1 minute.
2. Centrifuge at maximum speed for 3 minutes, open the tube and remove the supernatant carefully as much as possible.
3. Add 1 ml ddH_2O, invert the reaction tube three times and centrifuge at maximum speed for 3 minutes, open the tube and remove the supernatant carefully as much as possible.

   Note: Don't remove the pellet; it will be processed like the following steps!
4. Add **400 µl Lysis Solution GS** to the reaction tube containing the pellet. Dissolve the pellet by pipetting up and down several times. Try to avoid thereby the formation of air bubbles!

   Note: Optional add 10 µl Carrier RNA to the sample after adding Lysis Solution GS. See → "Intended use" p. 3 if Carrier RNA is necessary to add or not!
5. Add **50 µl Proteinase K** to the reaction tube and mix vigorously by pulsed vortexing for 10 seconds. Incubate at 70 °C for 15 minutes under continuously shaking at 1,000 rpm.

   Note: We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3-4 times during incubation. No shaking will reduce the lysis efficiency. After lysis centrifuge the tube shortly to remove condensate from the lid of the tube.
6. Add **200 µl Binding Solution VL** to the lysed sample, mix by pipetting up and down several times.

   Note: The Binding Solution VL is very viscously, please pipette carefully. It is important that the sample and the Binding Solution VL are mixed vigorously to get a homogeneous solution and avoid the formation of air bubbles.

7. Apply **the whole sample** to the Spin Filter located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

8. Open the Spin Filter and add **500 µl Washing Solution GS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

9. Open the Spin Filter and add **650 µl Washing Solution GS,** close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

10. Open the Spin Filter and add **650 µl Washing Solution BS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

11. Centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

12. Place the Spin Filter into a 1.5 ml Elution Tube. Open the cap of the Spin Filter and add **50 µl RNase-free Water** (prewarmed at 70 °C). Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Two elution steps with equal volumes of RNase-free Water (e.g. 30 µl + 30 µl) might increase the yield of extracted free-circulating DNA.

Note

The free-circulating DNA can be eluted with a lower (minimum 30 µl) or a higher volume of RNase-free Water (depends on the expected yield of free-circulating DNA). Elution with lower volumes of RNase-free Water increases the final concentration of free-circulating DNA. Store the extracted free-circulating DNA at 4–8 °C. For long time storage placing at -22 to -18 °C is recommended.

15.2 Protocol 5: Isolation of free-circulating DNA from serum, plasma, or other cell-free body fluids (except urine) of 2 ml up to 5 ml

Important

Pre-fill the needed amount of RNase-free Water in a 1.5 ml reaction tube and incubate the RNase-free Water at 70 °C until the elution step.

1. Add **100 µl of Enrichment Reagent VCR-1** and the sample into a 15 ml reaction tube and vortex shortly. Add **600 µl of Enrichment Reagent VCR-2** to the tube, mix shortly by vortexing. Incubate at room temperature for 10 minutes.
2. Centrifuge the tubes at least at 4,200 x g (~5,000 rpm) for 10 minutes, open the tube and remove the supernatant carefully as much as possible.
3. Add 5 ml ddH_2O to the tube, invert the tube three times and centrifuge at least at 4,200 x g (~5,000 rpm) 5 minutes, open the tube and remove the supernatant carefully as much as possible.

   Note: Don’t remove the pellet; it will be processed like the following steps!
4. Add **600 µl Lysis Solution GS** to the 15 ml reaction tube containing the pellet. Dissolve the pellet by pipetting up and down several times and **transfer the suspension into a 1.5 ml reaction tube**. Try to avoid thereby the formation of air bubbles!

   Note: Optional add 10 µl Carrier RNA to the sample after adding Lysis Solution GS. See → "Intended use" p. 3 if Carrier RNA is necessary to add or not!
5. Add **50 µl Proteinase K** to the reaction tube and mix vigorously by pulsed vortexing for 10 seconds. Incubate at 70 °C for 15 minutes under continuously shaking at 1,000 rpm.

   Note: We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3-4 times during incubation. No shaking will reduce the lysis efficiency.
6. Centrifuge at maximum speed for 2 minutes, open the tube and **transfer the supernatant** carefully in a new reaction tube.

   Note: If there is a pellet, don’t destroy it and pipette as much as possible from supernatant!

7. Add **300 µl Binding Solution VL** to the lysate, mix by pipetting up and down several times.

   Note: The Binding Solution VL is very viscously, please pipette carefully. It is important that the sample and the Binding Solution VL are mixed vigorously to get a homogeneous solution and avoid the formation of air bubbles.

8. Apply **600 µl of the sample** to the Spin Filter located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

9. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube and load the residual sample onto the Spin Filter. Close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

10. Open the Spin Filter and add **500 µl Washing Solution GS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

11. Open the Spin Filter and add **650 µl Washing Solution GS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

12. Open the Spin Filter and add **650 µl Washing Solution BS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

13. Centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

14. Place the Spin Filter into a 1.5 ml Elution Tube. Open the cap of the Spin Filter and add **50 µl RNase-free Water** (prewarmed at 70 °C). Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Two elution steps with equal volumes of RNase-free Water (e.g. 30 µl + 30 µl) might increase the yield of extracted free-circulating DNA.

Note

The free-circulating DNA can be eluted with a lower (minimum 30 µl) or a higher volume of RNase-free Water (depends on the expected yield of free-circulating DNA). Elution with lower volumes of RNase-free Water increases the final concentration of free-circulating DNA. Store the extracted free-circulating DNA at 4–8 °C. For long time storage placing at -22 to -18 °C is recommended.

15.3 Protocol 6: Isolation of free-circulating DNA from urine sample of 5 ml up to 10 ml

Important

Pre-fill the needed amount of RNase-free Water in a 1.5 ml reaction tube and incubate the RNase-free Water at 70 °C until the elution step.

Urine samples content cellular materials and cellular nucleic acids. In order to enrich only free-circulating DNA from the urine sample it is recommended to centrifuge the urine sample at maximum speed (e.g. 16,000 x g) and work subsequently only with the supernatant.

1. Add **100 µl of Enrichment Reagent VCR-1** and the sample into a 15 ml reaction tube and vortex shortly. Add **600 µl of Enrichment Reagent VCR-2** to the tube, mix shortly by vortexing. Incubate at room temperature for 10 minutes.
2. Centrifuge the tubes at least at 4,200 x g (~5,000 rpm) for 10 minutes, open the tube and remove the supernatant carefully as much as possible.
3. Add 5 ml ddH_2O to the tube, invert the tube three times and centrifuge at least at 4,200 x g (~5,000 rpm) 5 minutes, open the tube and remove the supernatant carefully as much as possible.

   Note: Don't remove the pellet; it will be processed like the following steps!
4. Add **600 µl Lysis Solution GS** to the 15 ml reaction tube containing the pellet. Dissolve the pellet by pipetting up and down several times and **transfer the suspension into a 1.5 ml reaction tube**. Try to avoid thereby the formation of air bubbles!

   Note: Optional add 10 µl Carrier RNA to the sample after adding Lysis Solution GS. See → "Intended use" p. 3 if Carrier RNA is necessary to add or not!
5. Add **50 µl Proteinase K** to the reaction tube and mix vigorously by pulsed vortexing for 10 seconds. Incubate at 70 °C for 15 minutes under continuously shaking at 1,000 rpm.

   Note: We recommend to use a shaking platform (thermal mixer, water bath or another rocking platform) for a continuous shaking of the sample. Alternatively, vortex the sample 3-4 times during incubation. No shaking will reduce the lysis efficiency.
6. Centrifuge at maximum speed for 2 minutes, open the tube and **transfer the supernatant** carefully in a new reaction tube.

   Note: If there is a pellet, don't destroy it and pipette as much as possible from supernatant!

7. Add **300 µl Binding Solution VL** to the lysate, mix by pipetting up and down several times.

   Note: The Binding Solution VL is very viscously, please pipette carefully. It is important that the sample and the Binding Solution VL are mixed vigorously to get a homogeneous solution and avoid the formation of air bubbles.

8. Apply **600 µl of the sample** to the Spin Filter located in a 2.0 ml Receiver Tube. Close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

9. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube and load the residual sample onto the Spin Filter. Close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute.

   Note: If the solution has not completely passed through the Spin Filter, centrifuge again at higher speed or prolong the centrifugation time.

   Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

10. Open the Spin Filter and add **500 µl Washing Solution GS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

11. Open the Spin Filter and add **650 µl Washing Solution GS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

12. Open the Spin Filter and add **650 µl Washing Solution BS**, close the cap and centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Discard the Receiver Tube with the filtrate. Place the Spin Filter into a new 2.0 ml Receiver Tube.

13. Centrifuge at maximum speed for 3 minutes to remove all traces of ethanol. Discard the 2.0 ml Receiver Tube.

14. Place the Spin Filter into a 1.5 ml Elution Tube. Open the cap of the Spin Filter and add **50 µl RNase-free Water** (prewarmed at 70 °C). Incubate at room temperature for 2 minutes. Centrifuge at 11,000 x g (~12,000 rpm) for 1 minute. Two elution steps with equal volumes of RNase-free Water (e.g. 30 µl + 30 µl) might increase the yield of extracted free-circulating DNA.

Note

The free-circulating DNA can be eluted with a lower (minimum 30 µl) or a higher volume of RNase-free Water (depends on the expected yield of free-circulating DNA). Elution with lower volumes of RNase-free Water increases the final concentration of free-circulating DNA. Store the extracted free-circulating DNA at 4–8 °C. For long time storage placing at -22 to -18 °C is recommended.

16 Troubleshooting

Problem / probable cause	Comments and suggestions
No pellet after first centrifugation step	
• Insufficient addition of VCR-1 or VCR-2	Make sure that both VCR-1 and VCR-2 are added to the reaction tube. Make sure that the right volume of VCR-1 and VCR-2 are added.
• Insufficient centrifugation	Make sure that centrifugation steps are carried out as describe in the manual. Otherwise repeat centrifugation.
• Removing of pellet	Ensure that the pellet is not discarded during removing the supernatant. In some cases the pellet is not been seen until the supernatant is removed completely.
Pellet is difficult to dissolve	
• Too much addition of VCR-1 or VCR-2	Make sure that both VCR-1 and VCR-2 are added as described in protocol.
• Lysis solution not enough added to pellet	Ensure that lysis solution is pipette as described in protocol.
• Pipette tip is clogged while dissolving the pellet	Cut the slide edge of pipette tip and try to transfer the pellet as much as possible.
Clogged Spin Filter	
• Insufficient lysis and/or too much starting material	Increase lysis time. Increase centrifugation speed. After lysis centrifuge the lysate to pellet unlysed material. Reduce amount of starting material.
Low amount of extracted free-circulating DNA	
• Insufficient lysis	Increase lysis time. Reduce amount of starting material. Overloading of Spin Filter reduces yield!
• Incomplete elution	Prolong the incubation time with RNase-free Water to 5 minutes or repeat elution step once again. Take a higher volume of RNase-free Water.

• Insufficient mixing with Binding Solution	Mix sample with Binding Solution by pipetting or by vortexing prior to transfer of the sample onto the Spin Filter.
Low concentration of extracted free-circulating DNA	
• Too much RNase-free Water	Elute the free-circulating DNA with lower volume of RNase-free Water.
• No Carrier RNA added	Add Carrier RNA to the sample, as described in the manual above.

17 Related Products

Name	Amount	Order No.
Products for Epigenetics		
innuCONVERT Bisulfite Basic Kit	8 rxn	845-IC-1000008
	40 rxn	845-IC-1000040
	80 rxn	845-IC-1000080
innuCONVERT Bisulfite All-In-One Kit	8 rxn	845-IC-2000008
	40 rxn	845-IC-2000040
	80 rxn	845-IC-2000080
Products for PCR and Real-Time PCR		
innuTaq Hot-A DNA Polymerase [5 U/µl]	500 Units	845-EZ-3000500
50x inNucelotide Mix (12.5 mM)	2x 0.5 ml	845-AS-9000100
innuMIX qPCR MasterMix Probe	100 rxn	845-AS-1200100
	200 rxn	845-AS-1200200
innuMIX qPCR MasterMix SyGreen	100 rxn	845-AS-1300100
	200 rxn	845-AS-1300200

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