For research use only. Not for use in diagnostic procedures.

chemagic cfDNA 1.5k Kit H96

(art. No. CMG-1396)

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Any further questions?
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Intended Use

With the **chemagic cfDNA 1.5k Kit H96** circulating cell-free DNA (cfDNA) can be isolated from 0,5 – 1,5 mL of plasma obtained from human whole blood samples. There are two protocol versions given, one for the extraction of sample volumes from 0,5 - 0,75 mL (see page 7) and one for the extraction of sample volumes from 1,0 - 1,5 mL (see page 11). For sample volumes higher than 0,75 mL and smaller than 1,0 mL the 1,0 - 1,5 mL protocol has to be used with samples filled up to 1,5 mL. All the reagents needed for the isolation of cfDNA are included in the kit, with the exception of nuclease-free water for dissolving **Proteinase K** and PBS or isotonic NaCl solution to fill up plasma samples to 0,75 mL per plate. The kit components (reagents and plastic ware) provide sufficient material for 960 preparations. The kit is designed to be used with the **chemagic 360** and integrated **chemagic Dispenser**. The product is intended for professional users such as technicians and physicians trained in molecular biology techniques.

Contents of the Kit

M-PVA Magnetic Beads	1 x 95 mL	**Wash Buffer 3**	1 x 1,5 L
Lysis Buffer 1	1 x 80 mL	**Wash Buffer 4**	1 x 1,5 L
Proteinase K (lyophilized)	38 vials	**Wash Buffer 6**	1x 1,0 L
Poly(A) RNA (lyophilized)	10 vials	**Elution Buffer 5**	1 x 100 mL
Poly(A) RNA Buffer	10 vials	**Deep Well Plate**	70 Plates
Binding Buffer 2	1 x 2,5 L	**Disposable Tips**	960

Functional Principle

The **chemagic cfDNA 1.5k Kit H96** is based on chemagic Technology using **M-PVA Magnetic Beads** for the isolation of cfDNA. The cfDNA binds to paramagnetic beads which are magnetically separated from the sample material. During subsequent steps contaminants are removed and the purified cfDNA is transferred into an elution medium. The automated sample processing by the **chemagic 360** excludes cross contamination and ensures safe handling of infectious sample material.

Quality Control

Each lot is tested to ensure the product meets the defined specifications according to chemagen's quality management system. Suboptimal results may be obtained if the protocol is not strictly followed.

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Product Specifications

The kit is designed for the use with human plasma samples derived from EDTA, citrate or Streck Cell-free DNA BCT® tubes.

Fresh and frozen plasma can be used. Please refer to section "**Plasma Preparation Protocol**" below.

The kit is not intended for the use with whole blood or tissue sample material. The isolation efficiency of cfDNA with other sample materials has not been investigated.

All reagents required for the cfDNA isolation are included in the kit except nuclease-free water for dissolving **Proteinase K**.

The **Elution Buffer 5** included in this kit is 10 mM Tris-HCl pH 8,0 with 0,1 mM EDTA. 10 mM Tris-HCl pH 8,0 can also be used without any protocol adjustments. Water pH 8,0 may also be used, but the yield could be slightly decreased.

Required Equipment

- **chemagic 360** instrument (art. No. CMG-2024-0020) equipped with **chemagic 96 Rod Head Set** (art. No. CMG-370)
- 1 x **chemagic Lid-Set 6** (art. No. CMG-594)
- **chemagic Stand 2x12 (optional)** (art. No. CMG-300)

Stability and Storage

The shelf life of the kit is 18 months. Expiry dates are noted on the kit label and on the labels of the individual kit components. All components of the unused kit can be stored at room temperature. Do not use the kit beyond the expiry date.

Once the kit has been opened, the "in use" stability of the kit is 3 months. All **Wash Buffers**, **Lysis Buffer 1**, **Binding Buffer 2, Poly(A) RNA Buffer** and the **M-PVA Magnetic Beads** of the "in use" kits can be stored at room temperature.

Binding Buffer 2, **Wash Buffer 3** and **Wash Buffer 4** contain ethanol. Longer storage of the buffers without lids should be avoided. If ethanol evaporates the optimal yield cannot be guaranteed.

Store **Poly(A) RNA Buffer, Binding Buffer 2** and **Wash Buffer 3** in the dark.

Lysis Buffer 1 may form a precipitate upon storage. If necessary, warm up to 55 °C to dissolve.

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The reconstituted **Poly(A) RNA** in **Poly(A) RNA Buffer** and reconstituted **Proteinase K** are stable for 4 weeks at 4 °C.

For long-term storage we recommend to store the reconstituted **Poly(A) RNA** in **Poly(A) RNA Buffer** and **Proteinase K** in aliquots at -20 °C. Do not freeze the **Poly(A) RNA** in **Poly(A) RNA Buffer** and **Proteinase K** aliquots after thawing. Equilibrate Poly(A) RNA and Proteinase K to room temperature before use.

The use of **Poly(A) RNA** is recommended as indicated in the detailed protocol description below. The extraction of cfDNA using this kit is possible without the use of **Poly(A) RNA** however the quantification of cfDNA may vary.

Quantification Methods – Comments

In some cases you may find traces of **M-PVA Magnetic Beads** remaining in the eluate. In such a case we recommend a short centrifugation of the samples to isolate the remnant **M-PVA Magnetic Beads** at the bottom of the vessel, or perform an additional separation step using an appropriate **chemagic Stand** in order to separate traces of particles.

During development, the performance of this kit was evaluated using the following quantification methods:

- qPCR on ALU115 primer set[1] on the QuantStudio® 5 Real-Time PCR System
- Qubit™ 1X dsDNA HS Assay Kit on the Qubit® 3.0 Fluorometer.

cfDNA yields isolated from human plasma samples are typically in the range of 1 - 30 ng/mL of plasma and therefore critically low and maybe outside the detection parameters determined by spectrophotometric methods.

If quantification of the extracted cfDNA is required a PCR-based method (qPCR, ddPCR) is recommended.

When using fluorometric quantification methods the addition of **Poly(A) RNA** is essential for a reliable performance. Fluorometric analysis of eluates extracted without the use of **Poly(A) RNA** may lead to varying and decreased quantification data. Additionally, be careful in interpreting the results if fluorometric methods are used for quantification as not only cfDNA but the total DNA is measured.

[1] ALU115 primer sequences obtained from: T.B. Hao, British Journal of Cancer (2014) 111, 1482–1489

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Qualitative Methods – Comments

During development the performance of this kit was evaluated using the following qualitative method:

- DNA NGS 3K Assay with the LabChip® GX Touch/GXII Touch.

To assess fragment distribution of the extracted cfDNA, fragment analysis systems may be used. A major peak at around 150 - 170 bp is expected for high quality mononucleosomal cfDNA, in some cases a smaller peak at around 300 bp representing dinucleosomal cfDNA fragments is also present.

The use of fragment analysis systems for cfDNA quantification did not lead to reproducible results during kit development and is not recommended.

Marker peak areas (marker included in fragment analyzer kits) vary according to different extraction chemistries. Variations of marker peak areas within one fragment analysis run may result in miscalculations.

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Plasma Preparation Protocol

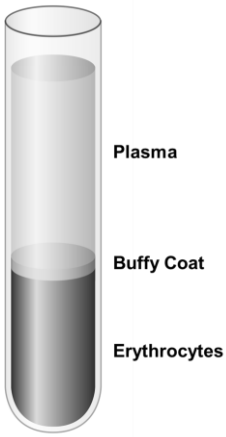
It is recommended to prepare plasma as fresh as possible (max. 5 days after blood draw). Longer storage of blood prior to plasma preparation may lead to poor separation results and higher background from genomic DNA.

A double centrifugation protocol during plasma preparation is recommended to minimize the potential for contamination of plasma with cells and genomic DNA. Transfer of any other blood components (buffy coat or red blood cells) should be avoided while separating the plasma fraction.

Please refer to the tube manufacturers specifications (max. centrifugation speed) for more information.

From 10 mL of whole blood approximately 4 - 5 mL plasma can be expected. Plasma volumes below 0,5 mL cannot be used for cfDNA extraction using this kit.

General Plasma Preparation Protocol



1. Centrifuge whole blood collection tube at 2.000 x g for 20 minutes.
2. Aspirate plasma carefully and at least 2 - 3 mm above the buffy coat layer, without disturbing the layer and transfer it into a new appropriate tube.
3. Centrifuge the plasma sample at 3.300 x g for 30 minutes.
4. Carefully transfer the supernatant to a fresh tube without disturbing the pellet, at least 2 - 3 mm above the pellet.
5. For direct use, storage of plasma sample at 2 - 8 °C for up to 10 hours is possible, for long-term storage -80 °C is recommended.
6. Before cfDNA extraction, equilibrate plasma to room temperature (19°C to 25°C). Thaw the frozen plasma storage tubes at room temperature for approximately 30 minutes (until thawed), or using a water bath (25°C to 30°C) for 10 minutes. Ensure that the tubes are thoroughly thawed before cfDNA extraction is started. If there are precipitates in the thawed plasma, dissolve them by inverting the tubes.

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Purification Protocol for 0,5 – 0,75 mL of Plasma Using the chemagic 360 with integrated chemagic Dispenser

Protocol name: **cfDNA 750 H96 360 drying prefilling VD200214.che**

Positioning Tips and Plates on the Tracking System for 0,5 - 0,75 mL of Plasma
Can be done manually or by an integrated robotic system

Position 1: Rack with Disposable Tips

Position 2: empty

! ***Up to 0,75 mL plasma has to be prepared in one Sample Plate (Position 3), more than 0,75 mL plasma has to be aliquoted in 2 Sample Plates (see protocol on page 11).***

Position 3: deep-well-plate (riplate SW) containing

0,75 mL sample
45 µL **Proteinase K**
0,6 µL **Poly(A) RNA**
38 µL **Lysis Buffer 1**
Binding Buffer 2 (added automatically)

! ***See "Processing Steps in Detail". Make sure to first add Proteinase K, then Poly(A) RNA followed by the Lysis Buffer 1, if you prepare a lysis premix.***

Position 4: empty deep-well-plate (riplate SW)
[**Wash Buffer 3** added automatically]

Position 5: empty deep-well-plate (riplate SW)
[**Wash Buffer 4** added automatically]

Position 6: deep-well-plate (riplate SW) prefilled with 45 µL **Magnetic Beads**
[**Wash Buffer 4** added automatically]

Position 7: deep-well-plate (riplate SW) prefilled with 50 - 100 µL **Elution Buffer 5**

Position 8: empty deep-well-plate (riplate SW)
[**Wash Buffer 6** added automatically]

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Processing Steps in Detail

Before You Start

- Dissolve lyophilized **Poly(A) RNA** by adding 440 µL of **Poly(A) RNA Buffer** to the **Poly(A) RNA** tube and mix thoroughly. For long-term storage, aliquots should be made and stored at -20 °C.
- Dissolve lyophilized **Proteinase K** in 1,25 mL nuclease-free water.

! ***See "Stability and Storage"***

- A homogenous suspension of the **M-PVA Magnetic Beads** must be ensured to guarantee the correct **M-PVA Magnetic Bead** concentration. Mix the bottle containing the **M-PVA Magnetic Beads** vigorously and check the bottom of the bottle for **M-PVA Magnetic Beads** sedimentation before dispensing. Otherwise optimal cfDNA extraction performance cannot be ensured.

Minimum Filling Volumes

The buffer levels in the containers connected to the **chemagic Dispenser** should not be lower than the values given in the following table. Please connect the buffers to the pumps according to the positions given in the table.

Buffer	Position	Minimum Filling Volume for 96 samples
Wash Buffer 6	1	150 mL
Binding Buffer 2	2	200 mL
Wash Buffer 3	3	200 mL
Wash Buffer 4	4	200 mL

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Protocol Steps

1. Select the protocol "**check manifolds 1 – 6**" and press the [**Insert IDs**] or - if the enhanced functions are deactivated - the [**Start**] button. A small volume of buffer will be dispensed by each manifold sequentially starting with first manifold used for this application.
   If one of the manifolds does not show the dispensing of buffer through all nozzles please use the corresponding priming protocol for this manifold.
   Performing several runs a day it is only necessary to check the manifolds once at the beginning of the day.
2. Select the protocol "**cfDNA 750 H96 360 drying prefilling VD200214.che**" and press the [**Insert IDs**] button. Follow the instructions as given in the **chemagic QA software**. If the enhanced functions are deactivated continue without pressing the [**Insert IDs**] button.
3. Use Disposable Tips according to the positions of the samples and place the Tip Rack in **position 1** on the tracking system.
4. Check the volumes in the buffer supply containers and confirm by pressing the [**OK**] button.

Take care that all buffer containers positioned on the plastic stand contain enough buffer. 96 isolations can only be performed if the buffer levels are not below the indicated minimum filling volume (see above "Minimum Filling Volume"). Otherwise replace with a new container and transfer the remaining buffer volumes into the new container.

5. Select the number of samples for prefilling by using the drop-down-menu. The scheme for positioning the samples will be shown after selecting. Take care to use the given positions! Confirm by pressing the [**OK**] button.
6. Prefill the **Elution Buffer 5** and the thoroughly suspended **M-PVA Magnetic Beads** according to the sample positions.

As a general rule, 5 - 10 µL of Elution Buffer 5 loss is expected during the run.

7. Prefill the thoroughly suspended **M-PVA Magnetic Beads** according to the sample positions.
8. Place the plates on the tracking system according to the instructions given by the **chemagic QA software**.

For indication of volumes and sample positions see "Positioning Tips and Plates on the Tracking System".

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9. Prefill the selected wells of the Sample Plate with 0,75 mL plasma equilibrated to room temperature. If less than 0,75 mL of plasma is available, fill the sample up to 0,75 mL with PBS or isotonic NaCl solution. Add 45 µL **Proteinase K**, 0,6 µL **Poly(A) RNA** and 38 µL **Lysis Buffer 1**.

   It is possible to premix **Proteinase K**, **Poly(A) RNA** and **Lysis Buffer 1** (choose the appropriate volume of **Proteinase K / Poly(A) RNA** / **Lysis Buffer 1** to ensure you have sufficient for the number of isolations).

! ***The Proteinase K activity will decrease after incubation longer than 10 minutes in Lysis Buffer 1. Ensure that all samples are mixed with* Proteinase K / Poly(A) RNA / Lysis Buffer 1 *within this time.***

10. Place the Sample Plate in **position 3** on the tracking system.
11. Check all plates and racks for accurate orientation and fitting.
12. Close the front door and start the process by pressing the [**Start**] button. Subsequently, the lysate will be mixed automatically.
13. After the isolation procedure has finished, use the [**Turn Table**] button to unload the tracking system. Each click on the [**Turn Table**] moves the tracking system (table) clockwise by one position.

! ***Never move the tracking system (table) manually. All movements have to be performed with the [Turn Table] function***.

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Purification Protocol for 1,0 – 1,5 mL of Plasma Using the chemagic 360 with integrated chemagic Dispenser

Protocol name: **cfDNA 1.5k H96 360 drying prefilling VD200213.che**

Positioning Tips and Plates on the Tracking System for 1,0 - 1,5 mL of Plasma
Can be done manually or by an integrated robotic system

Position 1: Rack with Disposable Tips

Position 2: deep-well-plate (riplate SW) containing

0,75 mL sample
45 µL **Proteinase K**
0,6 µL **Poly(A) RNA**
38 µL **Lysis Buffer 1**
Binding Buffer 2 (added automatically)

Position 3: deep-well-plate (riplate SW) containing

Components and volumes just as Position 2

! ***See "Processing Steps in Detail". Make sure to first add Proteinase K, then Poly(A) RNA followed by the Lysis Buffer 1, if you prepare a lysis premix.***

Position 4: empty deep-well-plate (riplate SW)
[**Wash Buffer 3** added automatically]

Position 5: deep-well-plate (riplate SW) prefilled with 45 µL **Magnetic Beads**
[**Wash Buffer 4** added automatically]

Position 6: deep-well-plate (riplate SW) prefilled with 45 µL **Magnetic Beads**
[**Wash Buffer 4** added automatically]

Position 7: deep-well-plate (riplate SW) prefilled with 50 - 100 µL **Elution Buffer 5**

Position 8: empty deep-well-plate (riplate SW)
[**Wash Buffer 6** added automatically]

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Processing Steps in Detail

Before You Start

- Dissolve lyophilized **Poly(A) RNA** by adding 440 µL of **Poly(A) RNA Buffer** to the **Poly(A) RNA** tube and mix thoroughly. For long-term storage aliquots should be made and stored at -20 °C.
- Dissolve lyophilized **Proteinase K** in 1,25 mL nuclease-free water.

! ***See "Stability and Storage"***

- A homogenous suspension of the **M-PVA Magnetic Beads** must be ensured to guarantee the correct **M-PVA Magnetic Bead** concentration. Mix the bottle containing the **M-PVA Magnetic Beads** vigorously and check the bottom of the bottle for **M-PVA Magnetic Beads** sedimentation before dispensing. Otherwise optimal cfDNA extraction performance cannot be ensured.

Minimum Filling Volumes

The buffer levels in the containers connected to the **chemagic Dispenser** should not be lower than the values given in the following table. Please connect the buffers to the pumps according to the positions given in the table.

Buffer	Position	Minimum Filling Volume for 96Samples
Wash Buffer 6	1	150 mL
Binding Buffer 2	2	250 mL
Wash Buffer 3	3	200 mL
Wash Buffer 4	4	200 mL

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   If one of the manifolds does not show the dispensing of buffer through all nozzles please use the corresponding priming protocol for this manifold.
   Performing several runs a day it is only necessary to check the manifolds once at the beginning of the day.
2. Select the protocol "**cfDNA 1.5k H96 360 drying prefilling VD200213.che**" and press the [**Insert IDs**] button. Follow the instructions as given in the **chemagic QA software**. If the enhanced functions are deactivated continue without pressing the [**Insert IDs**] button.
3. Use Disposable Tips according to the positions of the samples and place the Tip Rack in **position 1** on the tracking system.
4. Check the volumes in the buffer supply containers and confirm by pressing the [**OK**] button.

Take care that all buffer containers positioned on the plastic stand contain enough buffer. 96 isolations can only be performed if the buffer levels are not below the indicated minimum filling volume (see above "Minimum Filling Volume"). Otherwise replace with a new container and transfer the remaining buffer volumes into the new container.

5. Select the number of samples for prefilling by using the drop-down-menu. The scheme for positioning the samples will be shown after selecting. Take care to use the given positions! Confirm by pressing the [**OK**] button.
6. Prefill the **Elution Buffer 5** and the thoroughly suspended **M-PVA Magnetic Beads** according to the sample positions.

As a general rule, 5 - 10 µL of Elution Buffer 5 loss is expected during the run.

7. Prefill the thoroughly suspended **M-PVA Magnetic Beads** according to the sample positions.
8. Place the plates on the tracking system according to the instructions given by the **chemagic QA software**.

For indication of volumes and sample positions see "Positioning Tips and Plates on the Tracking System".

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9. Prefill the selected and corresponding wells of the 2 sample plates with 0,5 – 0,75 mL plasma equilibrated to room temperature. If less than 0,75 mL of plasma per plate is available, fill the sample up to 0,75 mL with PBS or isotonic NaCl solution. Make sure to split the plasma volume equally to both plates. Add 45 µL **Proteinase K**, 0,6 µL **Poly(A) RNA and** 38 µL **Lysis Buffer 1** to each sample well.

   It is possible to premix **Proteinase K**, **Poly(A) RNA** and **Lysis Buffer 1** (choose the appropriate volume of **Proteinase K** / **Poly(A) RNA** / **Lysis Buffer 1** to ensure you have sufficient for the number of isolations).

! ***The Proteinase K activity will decrease after incubation longer than 10 minutes in Lysis Buffer 1. Ensure that all samples are mixed with Proteinase K / Poly(A) RNA / Lysis Buffer 1 within this time.***

10. Place the Sample Plates in **position 2** and **position 3** on the tracking system.
11. Check all plates and racks for accurate orientation and fitting.
12. Close the front door and start the process by pressing the [**Start**] button. Subsequently, the lysate will be mixed automatically.
13. After the isolation procedure has finished, use the [**Turn Table**] button to unload the tracking system. Each click on the [**Turn Table**] moves the tracking system (table) clockwise by one position.

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Cleaning Information

Once per week - preferably before the weekend - clean the **chemagic Dispenser**. Select the protocol "**regular cleaning procedure.che**" and press the [**Insert IDs**] or the [**Start**] button if the enhanced functions are deactivated. Follow the instructions as given in the **chemagic QA software**. Prior to the next use of the **chemagic Dispenser** perform the appropriate priming protocol. The cleaning of the **chemagic Dispenser** with 70 % ethanol is recommended once per month, use the **"intensive cleaning procedure.che**" instead of the regular cleaning for this purpose.

! ***It is mandatory to perform the "regular cleaning procedure" if the chemagic Dispenser will not be used for prolonged period; this is to maintain the performance of the instrument when returning the instrument to service.***

Take care to drain the waste container frequently. Please consult local, state and federal regulations for additional guidance on disposal.

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Safety Information

To avoid injuries working with the kit components always wear safety glasses, disposable gloves and protective clothing. For detailed information please refer to the according Safety Data Sheet.

Reagent 1: **M-PVA Magnetic Beads** No hazardous substances in reportable concentrations, which must be labeled.
Reagent 2: **Lysis Buffer 1** **Substance(s):** Sodium dodecyl sulfate 20% ($CH_3(CH_2)_{11}OSO_3Na$) **CAS No.:** 151-21-3 ($CH_3(CH_2)_{11}OSO_3Na$)
Reagent 3: **Binding Buffer 2** **Substance(s):** Guanidine thiocyanate 35 - 45% ($C_2H_6N_4S$), dissolved in ethanol 20 - 40% (EtOH) **CAS No.:** 64-17-5 (EtOH), 593-84-0 ($C_2H_6N_4S$)
Reagent 4: **Wash Buffer 3** **Substance(s):** Guanidine thiocyanate 15 - 25 % ($C_2H_6N_4S$), dissolved in ethanol 30 - 50% (EtOH) **CAS No.:** 64-17-5 (EtOH), 593-84-0 ($C_2H_6N_4S$)
Reagent 5: **Wash Buffer 4** Substance(s): aqueous ethanol solution 70 - 90% (C_2H_5OH) **CAS No.:** 64-17-5 (EtOH)
Reagent 6: **Elution Buffer 5** No hazardous substances in reportable concentrations, which must be labelled.
Reagent 7: **Wash Buffer 6** No hazardous substances in reportable concentrations, which must be labelled.
Reagent 8: **Poly(A) RNA Buffer** **Substance(s):** Guanidine thiocyanate 30 - 40%($C_2H_6N_4S$) **CAS No.:** 593-84-0 ($C_2H_6N_4S$)
Reagent 9: **Poly(A) RNA** No hazardous substances in reportable concentrations, which must be labeled.

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