MANUAL

chemagic™ cfDNA 5k Kit H24

Product number: **CMG-1304**
Reagents for 240 extractions.

Version: 240119 EN

GTIN 4260543361440

Manufacturer: Revvity chemagen Technologie GmbH
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CONTENT OF THE KIT

Reagents	Plastic material
Magnetic Beads	chemagic Deep Well Plate 24 XL
Lysis Buffer 1	chemagic Tips XL
Binding Buffer 2	chemagic Elution Tube 13 mL
Wash Buffer 3	chemagic Elution Tube 4 mL
Wash Buffer 4	
Elution Buffer 5	
Proteinase K (lyophilized)	
Poly(A) RNA (lyophilized)	
Poly(A) RNA Buffer	

REQUIRED ITEMS

Item	Product no.
chemagic 360 instrument or	2024-0020
chemagic 360-D instrument	2024-0010
chemagic 24 Rod Head Set (supplied with the instrument)	CMG-376
chemagic 13 mL Double Bottom Rack H24	CMG-13005340

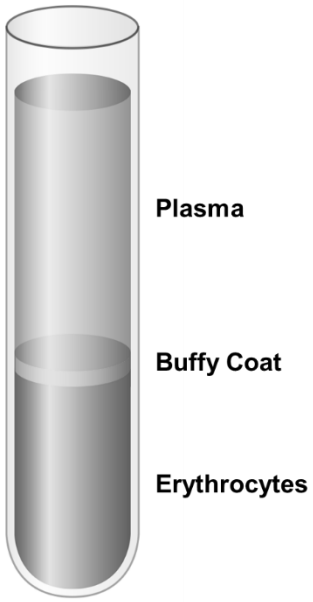
PLASMA PREPARATION PROTOCOL

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 3 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. 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.

PURIFICATION PROTOCOL FOR 3 - 5 ML OF HUMAN PLASMA USING THE CHEMAGIC 360 WITH INTEGRATED CHEMAGIC DISPENSER

Protocol name: chemagic cfDNA 3-5k 360 H24 prefilling VD190521.che

Positioning Tips and Plates on the Tracking System

Can be done manually or by an integrated robotic system.

Position	Material in position
Position 1	chemagic Tip Rack 24 with disposable chemagic Tips XL
Position 2	chemagic Deep Well Plate 24 XL (on chemagic 24 XL Adapter) containing:
	3 – 5 mL plasma
	150 µL Proteinase K
	1.3 µL Poly(A) RNA
	250 µL Lysis Buffer 1
	Binding Buffer 2 [added automatically]
	NOTE: See "Processing Steps".
Position 3	chemagic Elution Tubes 13 mL in chemagic 13 mL Double Bottom Rack H24 prefilled with 150 µL Magnetic Beads [Wash Buffer 3 added automatically]
Position 4	empty chemagic Elution Tubes 13 mL in chemagic 13 mL Double Bottom Rack H24 [Wash Buffer 4 added automatically]
Position 5	empty chemagic Elution Tubes 13 mL in chemagic 13 mL Double Bottom Rack H24 [Wash Buffer 4 added automatically]
Position 6	chemagic Elution Tubes 4 mL in chemagic Elution Rack 24 x 4 prefilled with 70 – 100 µL Elution Buffer 5
Position 7	empty chemagic Elution Tubes 13 mL in Elution Rack 24 x 13 [for used chemagic Tips XL] (following discarding of Disposable Tips the Tubes can be re-used for the same step)

DETAILED PROTOCOL DESCRIPTION

Protocol Procedure

The protocol is suitable for processing up to 24 samples in parallel (see “Processing Steps” below). For detailed instructions on the use of the chemagic 360 instrument, please refer to the chemagic 360 User Manual.

NOTE: Samples and reagents must be brought to room temperature (+19 to +25 °C) before use.

Connect the reagent bottles to the chemagic 360 instrument as follows:

Pump	Buffer
Pump 1	Binding Buffer 2
Pump 2	Wash Buffer 3
Pump 3	Wash Buffer 4
Pump 4	Not connected
Pump 5	Not connected
Pump 6	Not connected

NOTE: Recap the bottles tightly immediately after use or keep the bottles connected tightly to the chemagic 360 instrument. Binding Buffer 2, Wash Buffer 3 and Wash Buffer 4 contain ethanol. If ethanol evaporates, the optimal yield or detection sensitivity cannot be guaranteed.

Processing Steps

1. Check all kit components for integrity. In case of damage, contact your supplier.
2. Before prefilling the plates mark each plate with material in position (samples, Magnetic Beads and buffers).
3. Reconstitute the Proteinase K and Poly(A) RNA:

Component	Reconstitution
Proteinase K	Add molecular biology grade water to Proteinase K bottle and mix gently until dissolved (volume see label).
Poly(A) RNA	Dissolve lyophilized Poly(A) RNA by adding Poly(A) RNA Buffer to the Poly(A) RNA tube and mix thoroughly (volume see label).

4. Fill and prime the chemagic 360 tubing with reagents by choosing the protocol "**prime manifolds H24 all 360 V150116.che**". Press [Insert IDs], follow the instructions given in the chemagic QA software and start priming by pressing [OK]. If functions enabling the ID data input are deactivated, start priming directly by pressing [Start].

NOTE: Priming needs to be done when reagent bottles are connected to the chemagic 360 instrument for the first time or when the instrument's tubing is not already filled with the above-mentioned reagents.

5. If priming is not needed, select the protocol "**check manifolds H24 all 360 V150116.che**" and press [Insert IDs] or - if the enhanced functions are deactivated - [Start]. A small volume of buffer will be dispensed by each pump sequentially starting with the first pump used for this application. If one of the pumps does not show dispensing of buffer through all nozzles, please use the corresponding priming protocol for this pump. Performing several runs a day it is only necessary to check the pumps once at the beginning of the day.
6. Select the protocol "**chemagic cfDNA 3-5k 360 H24 prefilling VD190521.che**" and press [Insert IDs] and follow the instructions given in the chemagic QA software.
7. Ensure chemagic Tip Rack 24 contains enough tips and is aligned with the positions of the samples and place the chemagic Tip Rack 24 with chemagic Tips XL in position 1 on the tracking system.
8. Check the volumes in the buffer supply containers and confirm by pressing [OK].

NOTE: Take care that all buffer containers positioned on the plastic stand contain enough buffer. 24 isolations can only be performed if the buffer levels are not below the indicated minimum filling volume (see "Minimum filling volumes"). Otherwise replace with a new container and transfer the remaining buffer volumes into the new container.

9. 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 [OK].
10. Prefill the selected wells of the sample plate with up to 5 mL sample. To ensure the homogeneity of the samples, mix the samples gently prior to pipetting in the wells of the sample plate.

11. Prefill the Elution Buffer 5 and the thoroughly resuspended Magnetic Beads by pipetting manually according to each corresponding well in use.

Component	Plate position on chemagic 360 instrument	Volume/ well
Magnetic Beads	3	150 µL
Elution Buffer 5	6	70 – 100 µL

NOTE: The Magnetic Bead suspension should be mixed vigorously before dispensing; otherwise, the suspension is not homogenous, and the DNA yield could be low.

12. Add the following reagents to the wells containing sample:
    - 150 µL Proteinase
    - 1.3 µL Poly(A) RNA
    - 250 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 volume for the number of isolations). Make sure to first add Proteinase K, then Poly(A) RNA followed by Lysis Buffer 1 when preparing a premix.

NOTE: 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.

13. Place the chemagic Deep Well Plate 24 XL on the tracking system according to the instructions given by the chemagic QA software.
14. Place the sample plate in position 2 on the tracking system.
15. Check all plates for accurate orientation and fitting.
16. Close the front door and start the process by pressing [Start].
17. The automated cfDNA extraction process is initiated.
18. After the isolation procedure has finished use the [Turn Table] button to unload the tracking system. Each click on [Turn Table] moves the tracking system (table) clockwise by one position.

ATTENTION! Never move the tracking system (table) manually. This might damage the instrument. All movements must be performed with the [Turn Table] function.

NOTE: Opening the chemagic 360 instrument door while the automated extraction run is ongoing, will terminate the run and the samples in process may be lost.

For information on cleaning the instrument see section “Cleaning and Maintenance”.

CLEANING AND MAINTENANCE

Cleaning and maintenance of the system is described in detail in the chemagic 360 User Manual. The system cleaning is performed once per week. Clean the chemagic Dispenser as follows.

- Select the protocol "**regular cleaning procedure 24 dispenser 360 V150116.che**" and press [Insert IDs] or [Start] if the enhanced functions are deactivated. Follow the instructions as given in the 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. Simply use the "**intensive cleaning procedure H24 dispenser 360 V150116.che**" instead of the regular one for this purpose.
- If the chemagic Dispenser will not be used for a longer time, it is mandatory to perform the "regular cleaning procedure" to maintain the performance of the instrument when bringing it back into service.
- Take care to drain the waste container frequently. Please consult local, state, and federal regulations for additional guidance on disposal.

MINIMUM FILLING VOLUMES

The buffer levels in the containers connected to the chemagic Dispenser should not fall below the values given in the following table:

Buffer	**Position**	**Minimum filling volume for 24 Samples**
Binding Buffer 2	1	250 mL
Wash Buffer 3	2	150 mL
Wash Buffer 4	3	150 mL

ADDITIONAL INFORMATION

Safety Information

To avoid injuries when working with the kit components, always wear safety glasses, disposable gloves, and protective clothing. For detailed information, please refer to the corresponding safety data sheets (SDS).

Storage Conditions

All kit components can be stored at room temperature, except the reconstituted Proteinase K and Poly(A) RNA.

Store reconstituted Proteinase K at +2 to +8 °C. The reconstituted Proteinase K is stable for 28 days at +2 to +8 °C. For long term storage we recommend to store the reconstituted Proteinase K in aliquots at - 20 °C. Do not freeze the Proteinase K aliquots after thawing.

Store reconstituted Poly(A) RNA at +2 to +8 °C. The reconstituted Poly(A) RNA is stable for 28 days at +2 to +8 °C.

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.

For long-term storage we recommend storing 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. Before using equilibrate Poly(A) RNA and Proteinase K to room temperature.

The use of Poly(A) RNA is recommended. The extraction of cfDNA using this kit is possible without the use of Poly(A) RNA however the quantification of cfDNA may vary.

GENERAL REMARKS

The Elution Buffer 5 included in this kit is 10 mM Tris-HCl pH 8.0 with 0.1 mM EDTA. TE buffer 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.

The Magnetic Bead suspension should be mixed vigorously before dispensing, otherwise the suspension is not homogenous, and the NA yield could be low.

Expiry dates are stated on the box of the kit. Do not use any component of the kit beyond the expiration date.

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”.
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.

QUANTIFICATION METHODS

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

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

- qPCR on ALU115 primer set 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.

QUALITATIVE METHODS

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.

WARRANTY

Any change or modification of the procedure not recommended by the manufacturer may affect the results, in which event Revvity chemagen Technologie GmbH and its affiliates disclaim all warranties expressed, implied or statutory including the implied warranty of merchantability and fitness for use.

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