MANUAL

chemagic™ cfDNA 5k Kit

Product number: **CMG-134**
Reagents for 40 extractions.

Version: 240215 EN

GTIN 4260543365080

Manufacturer: Revvity chemagen Technologie GmbH
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www.revvity.com

CONTENT OF THE KIT

Reagents
Magnetic Beads
Lysis Buffer 1
Binding Buffer 2
Wash Buffer 3
Wash Buffer 4
Elution Buffer 5
Proteinase K (lyophilized)
Poly(A) RNA (lyophilized)
Poly(A) RNA Buffer

REQUIRED ITEMS

Item	Product no.
chemagic Stand 2x12 for 1.5 mL and 2.0 mL tubes	CMG-300
chemagic Magnetic Stand Type F for 50 mL centrifuge tubes	CMG-302
chemagic cfDNA stand 12 for 50 mL centrifuge tubes (optional) (This stand allows for the simultaneous separation of 12 samples and is recommended for faster processing of multiple samples)	CMG-306
(Thermo-) shaker suitable for 50 mL tubes	-
Vortex mixer	-
Thermoshaker for 1.5 mL tubes	-
Conical 50 mL tubes	-
1.5 mL low binding tubes	-
Centrifuge for 50 mL tubes (optional)	-
Antofoam A Concentrate (optional)	Sigma, Product number A5633

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.

General Plasma Preparation Protocol

1. Centrifuge whole blood collection tube at 2.000 x g for 20 min.
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 min.
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.

Before cfDNA extraction, equilibrate plasma to room temperature. Thaw the frozen plasma storage tubes at room temperature for approximately 30 min (until thawed) or using a water bath (25 °C to 30 °C) for 10 min. 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 UP TO 5 ML OF HUMAN PLASMA

Functional Principle

The chemagic cfDNA 5k Kit is based on chemagic technology using Magnetic Beads for the isolation of cfDNA (Figure 1). 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.

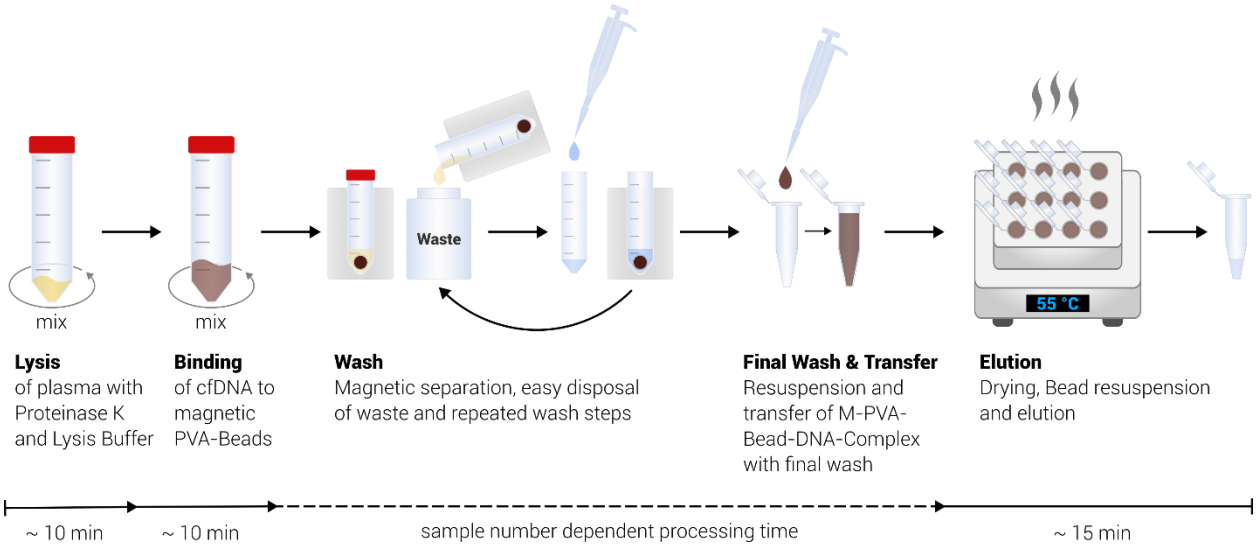

Figure 1. Functional principle of the CMG-134 cfDNA extraction kit.

DETAILED PROTOCOL DESCRIPTION

Protocol Procedure

Different buffer- and reagent volumes are required for different sample volumes, as shown in the table below.

Component	1 – 2 mL Plasma*	3 – 5 mL Plasma
Poly(A) RNA	1.3 µL	1.3 µL
Proteinase K	100 µL	150 µL
Lysis Buffer 1	100 µL	250 µL
Magnetic Beads	60 µL	150 µL
Binding Buffer 2	3 mL	6.25 mL
Drying Step	2 min at 55 °C	5 min at 55 °C
Elution Buffer 5	30 - 60 µL	50 - 100 µL

*For plasma volumes < 1 mL, top up to 1 mL with PBS and follow the recommended buffer and reagent volumes.

Processing Steps

1. Check all kit components for integrity. In case of damage, contact your supplier.
2. 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).

1. Preheat thermoshaker for 1.5 mL tubes to 55 °C.

2. Prefill the 50 mL sample tube with 5 µL Antifoam A concentrate (optional) and the indicated volume of Proteinase K, 1.3 µL dissolved Poly(A) RNA and Lysis Buffer 1. Add the plasma sample equilibrated to room temperature. Vortex briefly for 10 seconds and incubate the sample for 10 min at room temperature while mixing on a (thermo-) shaker (300 rpm).

NOTE: To avoid foaming and facilitate easy removal of supernatant with chemagic Magnetic Stands, the use of antifoam from the recommended manufacturer is advised. Alternatively, follow recommended steps below to remove foam.

NOTE: 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. Do not vortex the premix as this leads to foaming. Mix by gentle pipetting.

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.

3. Add the indicated volume of Magnetic Beads and Binding Buffer 2 to each sample (see Protocol Procedure). Vortex briefly and incubate for 10 min while mixing on a (thermo-) shaker (300 rpm) at room temperature.

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

4. Following binding incubation, place the tube on the chemagic Magnetic Stand Type F for 5 minutes and, if necessary, until the solution clears, and Magnetic Beads are completely separated. If necessary, remove any foam by pipetting (when no antifoam was used). Afterwards, discard the supernatant by pouring the tube now fixed within the chemagic Magnetic Stand Type F (Figure 1).

   Alternatively: Briefly centrifuge (1 min, 4000 rpm) the lysis/binding tube to remove any foam (when no antifoam was used) and to separate the Magnetic Beads. Keep the tube on the magnet for another minute and discard the supernatant by pouring as described previously.

   Multi-sample processing with the chemagic cfDNA stand 12 (CMG-306): Following binding incubation, unscrew the lid of the 50 mL tubes and place all tubes on the chemagic cfDNA stand 12 for 5 min and if necessary until the solution clears, and Magnetic Beads are completely separated. If necessary, remove any foam by pipetting (when no antifoam was used). Afterwards, transfer the individual tubes to the chemagic Magnetic Stand Type F, separate for 30 seconds and discard the supernatant by pouring the tube now fixed within the chemagic Magnetic Stand Type F.

NOTE: All following wash steps can be performed in the same manner.

5. Resuspend Magnetic Beads in 2 mL of Wash Buffer 3 by vortexing (10 – 30 sec) and place the tube on the chemagic Magnetic Stand Type F for 30 sec or until the solution clears and Magnetic Beads are completely separated. Discard the Wash Buffer 3 supernatant by pouring as described previously.
6. Remove the tube from the magnetic separator and repeat step 4 (vortex for 10 sec).
7. Repeat the washing procedure (step 5) using 2 mL Wash Buffer 4 once (vortex for 10 sec).
8. Remove the tube from the magnet position. Resuspend the beads in 600 µL Wash Buffer 4 by pipetting, then transfer the Magnetic Beads/DNA Complex to a 1.5 mL tube in the chemagic Stand 2x12 (non-magnet position). Use an additional 600 µL of Wash Buffer 4 to rinse the 50 mL tube and transfer any residual beads to the corresponding 1.5 mL tube and mix gently by pipetting 10 times. Place the 1.5 mL tube into the magnet position for at least 90 sec. Aspirate and discard the supernatant by pipetting (use 200 µL pipette tip to remove last traces).

NOTE: Make sure that there is no residual liquid and bead slurry left in the lid, alternatively liquid can be shortly spun down.

9. After removing the last traces of the supernatant, dry the bead pellet for the indicated time (see Protocol Procedure) at 55 °C in a thermoshaker (open tube lid).

NOTE: Make sure that the bead pellet is dried, extend drying time by another 1 – 2 min if necessary. The dried bead pellet should have a matte surface but no cracks (Figure 2).

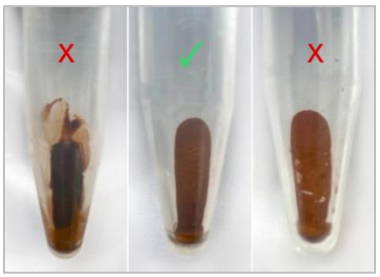

Figure 2. Bead pellet drying stages (left to right): the bead pellet is still wet and smears when the tube is twisted (x); dry bead pellet with a matte surface and slightly lighter color (✓); bead pellet with cracks should be avoided (x).

10. Add desired volume of Elution Buffer to the tube (see Protocol Procedure) and thoroughly resuspend the Magnetic Beads/DNA Complex by pipetting (10 - 15 times).
11. Incubate the suspension for 5 min at 55 °C with gentle agitation in a thermoshaker (500 rpm).

Following DNA elution, place the tube in a chemagic Stand 2x12 (magnet position) for 2 min or until all the Magnetic Beads have separated from the eluate. Transfer the eluate containing the purified cfDNA to a clean low binding tube.

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 “General 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.

QUANTIFICANTION 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[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.

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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