MANUAL

chemagic™ miRNA 200 Kit H96

Product number: **CMG-1224**
Reagents for 960 extractions.

Version: 240118 EN

GTIN 4260543365097

Manufacturer: Revvity chemagen Technologie GmbH
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CONTENT OF THE KIT

Reagents	Plastic material
Magnetic Beads	chemagic Tips 96 Racks
Lysis Buffer 1	chemagic Deep Well Plate 2 mL
Binding Buffer 2	chemagic Low Well Plates
Wash Buffer 3	
Wash Buffer 4	
Elution Buffer 5	
Proteinase K (lyophilized)	

REQUIRED ITEMS

Item	Product no.
chemagic 360 instrument or	2024-0020
chemagic 360-D instrument	2024-0010
chemagic 96 Rod Head Set (supplied with the instrument)	CMG-370

PURIFICATION PROTOCOL FOR 200 µL OF PLASMA USING THE CHEMAGIC 360 WITH INTEGRATED CHEMAGIC DISPENSER

Protocol name: chemagic miRNA 200 H96 drying prefilling VD210726.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 Tips 96 Rack (on special adapter)
Position 2	chemagic Low Well Plate (on special adapter) prefilled with 53 µL Magnetic Beads
Position 3	chemagic Deep Well Plate 2 mL (on special adapter) containing:
	200 µL plasma
	25 µL Proteinase K
	100 µL Lysis Buffer 1 [added automatically] and
	Binding Buffer 2 [added automatically]
	NOTE: See "Processing Steps".
Position 4	empty chemagic Deep Well Plate 2 mL (on special adapter) [Wash Buffer 3 added automatically]
Position 5	empty chemagic Deep Well Plate 2 mL (on special adapter) [Wash Buffer 4 added automatically]
Position 6	empty chemagic Deep Well Plate 2 mL (on special adapter) [Wash Buffer 4 added automatically]
Position 7	chemagic Deep Well Plate 2 mL (on special adapter) prefilled with 55 µL Elution Buffer 5
Position 8	empty

DETAILED PROTOCOL DESCRIPTION

Protocol Procedure

The protocol is suitable for processing up to 96 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	Not connected
Pump 2	Binding Buffer 2
Pump 3	Wash Buffer 3
Pump 4	Wash Buffer 4
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:

Component	Reconstitution
Proteinase K	Add molecular biology grade water to Proteinase K bottle and mix gently until dissolved (volume see label).

4. Fill and prime the chemagic 360 tubing with reagents by choosing the protocol "**prime manifolds H96 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 H96 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 miRNA 200 H96 drying prefilling VD210726.che**" and press [Insert IDs] and follow the instructions given in the chemagic QA software.
7. Ensure chemagic Tips 96 Rack contains enough tips and is aligned with the positions of the samples and place the chemagic Tips 96 Rack 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. 96 isolations can only be performed if the buffer levels are not below the indicated minimum filling volume (see below "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 200 µL sample. To ensure the homogeneity of the samples, mix the samples gently prior to pipetting in the wells of the sample plate.

11. Add the following reagents to the wells containing sample:
    - 25 µL Proteinase K
    - 100 µL Lysis Buffer 1

It is possible to premix Proteinase K and Lysis Buffer 1 (choose the appropriate volume of Proteinase K/ Lysis Buffer 1 to ensure you have sufficient volume for the number of isolations). To ensure the homogeneity of the samples, mix the samples gently prior to pipetting in the wells of the sample plate.

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/ Lysis Buffer 1 within this time

12. 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	2	53 µL
Elution Buffer 5	7	55 µL

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

13. Place the chemagic Deep Well Plates 2 mL on the tracking system according to the instructions given by the chemagic QA software.
14. Place the sample plate in position 3 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 miRNA 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 96 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 H96 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 96 Samples**
Binding Buffer 2	2	250 mL
Wash Buffer 3	3	150 mL
Wash Buffer 4	4	200 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.

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 storing the reconstituted Proteinase K in aliquots at - 20 °C. Do not freeze the Proteinase K aliquots after thawing.

Store Lysis Buffer 1 in the dark.

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

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.

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 miRNA 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.

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 Qubit™ microRNA Assay Kit on the Qubit® 3.0 Fluorometer.

miRNA yields isolated from human plasma samples are critically low and maybe outside the detection parameters determined by spectrophotometric methods. If quantification of the extracted miRNA is required a PCR-based method (qPCR, ddPCR) is recommended.

OPTIONAL

The addition of 5 µg glycogen (R0551, Thermo Fisher Scientific™, Waltham, MA, USA) to the lysates can be beneficial to increase miRNA yields but is not mandatory.

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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