MANUAL

chemagic™ RNA Tissue 10mg Kit H96

Product number: **CMG-1212**
Reagents for 960 extractions.

Version: 240115 EN

GTIN 4260543361259

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	
Wash Buffer 3	
Wash Buffer 4	
DNase I	
DNase I Buffer 5	
Elution Buffer 6	

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 10 MG OF TISSUE USING THE CHEMAGIC 360 WITH INTEGRATED CHEMAGIC DISPENSER

Protocol name: chemagic RNA Tissue 360 H96 drying prefilling VD210831.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 Deep Well Plate 2 mL (on special adapter) containing:
	Tissue homogenate
	600 µL Lysis Buffer 1
	Binding Buffer 2 [added automatically]
	NOTE: See "Processing Steps".
Position 3	chemagic Deep Well Plate 2 mL (on special adapter) prefilled with 70 µL Magnetic Beads [Wash Buffer 3 added automatically]
Position 4	empty chemagic Deep Well Plate 2 mL (on special adapter) [Wash Buffer 4 added automatically]
Position 5	chemagic Deep Well Plate 2 mL (on special adapter) prefilled with 600 µl DNase I Reaction Mix
	537 µL RNase free water
	60 µL DNase I Buffer 5
	3 µL DNase I
	Binding Buffer 2 (added automatically)
Position 6	empty chemagic Deep Well Plate 2 mL (on special adapter) [Wash Buffer 3 added automatically]
Position 7	empty chemagic Deep Well Plate 2 mL (on special adapter) [Wash Buffer 4 added automatically]
Position 8	chemagic Deep Well Plate 2 mL (on special adapter) prefilled with 50 - 100 µL Elution Buffer 6

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	Not connected
Pump 5	Wash Buffer 4
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.

Preparation of the Tissue Homogenate

1. Cut a tissue piece of up to 10 mg and transfer it into a process vessel which is suitable for the homogenization method of your choice (bead mill, rotor/stator, mortar/pestle etc.).
2. Add 600 µL Lysis Buffer 1 per 10 mg of tissue and homogenize the tissue. A good and thorough homogenization is the key for good yield and quality of the final RNA. After homogenization it might be necessary to centrifuge the samples to reduce the amount of foam and for removal of insoluble tissue debris.
3. Transfer the cleared supernatants into the chemagic Deep Well Plate 2 mL.
4. Depending on your homogenization method it might be necessary to additionally shear high molecular DNA – this can be done by vigorous mixing using a pipette.
5. Continue with Step 1 from “Processing Steps”

Preparation of Cell Pellets

1. Harvest the cells according to your specific SOP.
2. After centrifugation discard any liquid, resuspend the cells in 600 µL Lysis Buffer 1 and transfer the complete volume into the chemagic Deep Well Plate 2 mL.
3. Add 20 µL Proteinase K directly before starting the extraction on the instrument.
4. Continue with Step 1 from “Processing Steps”.

Preparation of White Blood Cells

1. Place 2 mL of fresh whole blood into a sterile 50 mL tube.
2. Add 15 mL Red Cell Lysis Buffer to the blood and invert the tube 4 times. Leave on bench for 10 minutes or until the suspension becomes translucent.

   (Red Cell Lysis Buffer can be ordered separately with order number: CMG-848)
3. Centrifuge at 3500 x g for 10 minutes to collect the white cells. Decant the supernatant and carefully aspirate the remaining supernatant from the top of the sample by pipetting. Be careful not to disturb the cell pellet.

   Exercise caution in pipetting to avoid loss of the white blood cell pellet.
4. Add 10 mL Red Cell Lysis Buffer and carefully wash remaining red cells on top of the white pellet without disturbing the white pellet.
5. Decant the supernatant and carefully aspirate the remaining supernatant from the top of the sample by pipetting.
6. Add 15 µL of a 1 M solution of TCEP and resuspend the pellet completely with 600 µL of Lysis Buffer.

   Alternatively: Resuspend the pellet completely with 600 µL of Lysis Buffer 1 and add 25 µL ß-Mercaptoethanol to the lysate.
7. This lysate can be frozen or directly used for extraction.
8. Transfer the complete volume into the chemagic Deep Well Plate 2 mL.
9. Continue with Step 1 from “Processing Steps”.

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.

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. Use of Proteinase K is recommended for the preparation of PAXgene™ Pellets and Cell Pellets and Proteinase K can be ordered separately with order number: CMG-835 (1.25 mL per vial, 20 mg/mL).
4. Reconstitute the Proteinase K:

Component	Reconstitution
DNase I	Dissolve the DNase I in the appropriate volume of sterile filtered 50 % glycerol/RNase free water solution (see flask label; RNase free water & glycerol are not provided in the kit).
Proteinase K	Add molecular biology grade water to Proteinase K bottle and mix gently until dissolved (volume see label).

5. 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.

6. 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.
7. Select the protocol "**chemagic RNA Tissue 360 H96 drying prefilling VD210831.che**" and press [Insert IDs] and follow the instructions given in the chemagic QA software.
8. Ensure chemagic Tips 96 Tray/ Rack contains enough tips and is aligned with the positions of the samples and place the chemagic Tips 96 Tray/ Rack in position 1 on the tracking system.
9. 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.

10. 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].
11. Prefill the Elution Buffer 6, DNase I Reaction Mix and the thoroughly resuspended Magnetic Beads by pipetting manually according to each corresponding well in use.

NOTE: For the DNase I Reaction Mix it is recommended to prepare a master mix.

Component	Plate position on chemagic 360 instrument	Volume/ well
Magnetic Beads	3	70 µL
DNase I Reaction Mix	5	600 µL
Elution Buffer 6	8	50–100 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. Place the chemagic Deep Well Plates 2 mL on the tracking system according to the instructions given by the chemagic QA software.
13. Place the sample plate in position 2 on the tracking system.
14. Check all plates for accurate orientation and fitting.
15. Close the front door and start the process by pressing [Start].
16. The automated RNA extraction process is initiated.
17. 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	250 mL
Wash Buffer 4	5	250 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 DNase I lyophilizate at +2 to +8 °C. After dissolving store DNase I at -15 to -20 °C.

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 7 included in this kit is 10 mM Tris-HCl pH 8.0. 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 DNA 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.

UV MEASUREMENTS/ REAL TIME PCR

In some cases, you may find some traces of Magnetic Beads left in the eluate. Such particles will not interfere with standard PCR and most downstream applications but may increase the background in UV measurements or could influence real time PCR.
In such a case we recommend performing an additional separation step using an appropriate chemagic magnetic stand to separate traces of particles.

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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