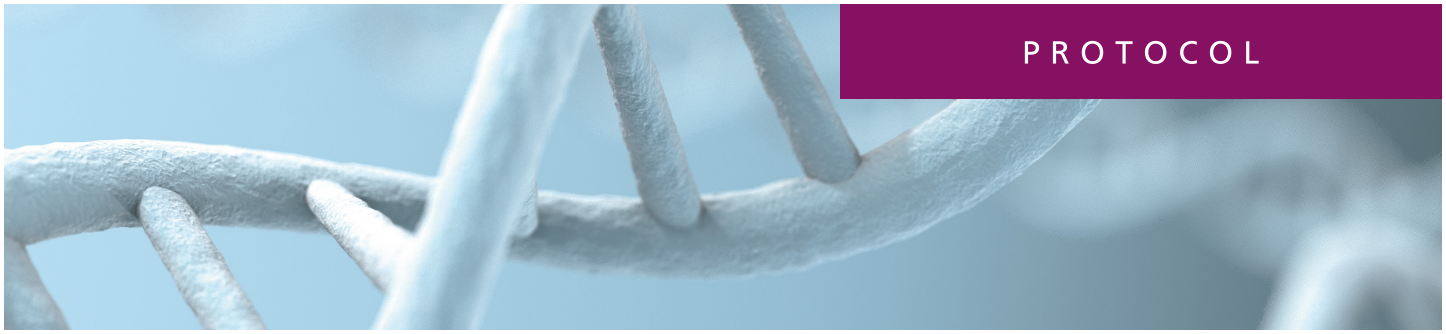

For research use only. Not for use in diagnostic procedures.

chemagic miRNA 200 H96 Kit

Intended Use

With the **chemagic miRNA 200 H96 Kit** miRNA can be isolated from 200 µL of plasma obtained from human whole blood samples. All the reagents needed for the isolation of small RNA are included in the kit, with the exception of nuclease-free water for dissolving Proteinase K. The kit components (reagents and plastic ware) provide sufficient material for 960 preparations. 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 65 mL	**Elution Buffer 5**	1 x 100 mL
Lysis Buffer 1	1 x 150 mL	**Low well Plates**	10 Plates
Proteinase K (lyophilized)	11 vials	**2 mL Deep Well Plates**	50 Plates
Binding Buffer 2	1 x 2,5 L	**Disposable Tips**	960
Wash Buffer 3	1 x 1,5 L		
Wash Buffer 4	1 x 1,5 L		

Functional Principle

The **chemagic miRNA 200 H96 Kit** is based on chemagic Technology using **M-PVA Magnetic Beads** for the isolation of miRNA. The miRNA binds to paramagnetic beads, which are magnetically separated from the sample material. During subsequent steps contaminants are removed and the purified miRNA 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.

Any further questions?

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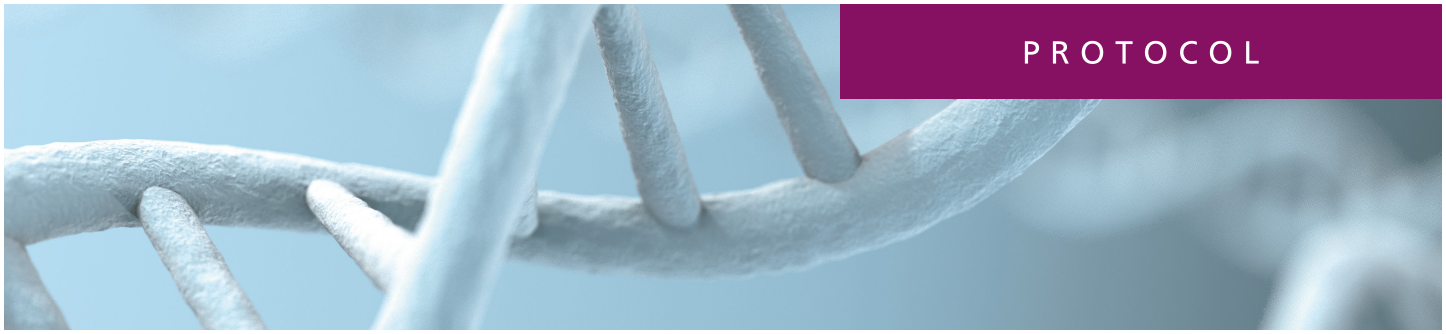

Product Specifications

The kit is designed for the use with human plasma samples. Fresh and frozen plasma can be used.

The isolation efficiency of miRNA with other sample materials has not been investigated.

All reagents required for the miRNA isolation are included in the kit except nuclease-free water.

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)

Optional

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

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**, **Binding Buffer 2** and the **M-PVA Magnetic Beads** of the “in use” kits can be stored at room temperature.

Lysis Buffer 1 must be stored in the dark. **Lysis Buffer 1** may form a precipitate upon storage. If necessary, warm up 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.

The reconstituted **Proteinase K** is stable for 4 weeks at 4 °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. Before use, equilibrate **Proteinase K** to room temperature.

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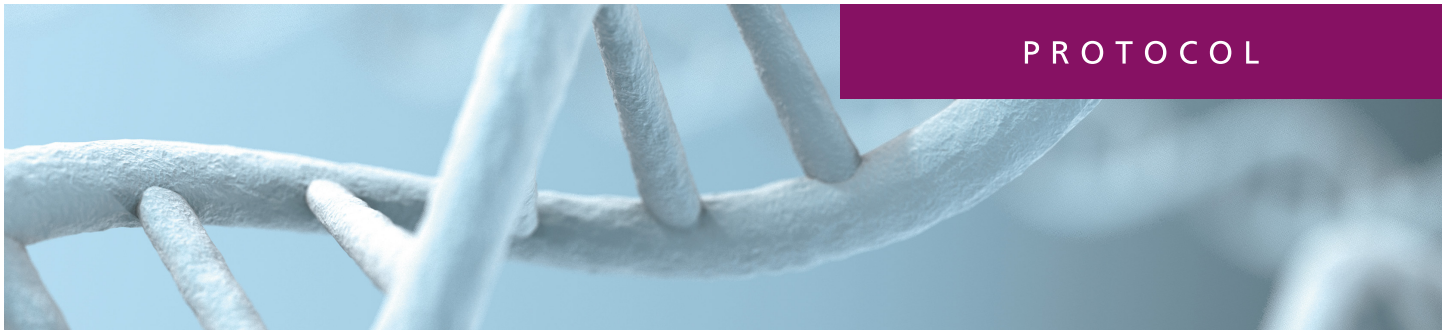

Purification Protocol for 200 µL of Plasma Using the chemagic 360

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 1: Rack with Disposable Tips

Position 2: low-well-plate (MICROTITER SYSTEM) prefilled with 53 µL **Magnetic Beads**

Position 3: deep-well-plate (riplate SW) prefilled with

200 µL plasma
100 µL **Lysis Buffer 1**
25 µL **Proteinase K**
1400 µL **Binding Buffer 2** (added automatically)

! ***See "Processing Steps in Detail".***

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

Position 7: deep-well-plate Well Plate (riplate SW) prefilled with
55 µL **Elution Buffer 5**

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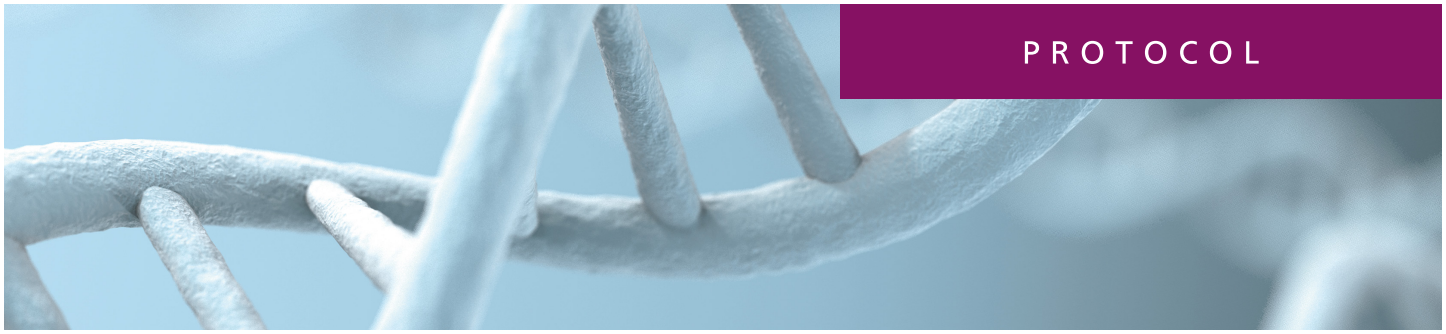

Processing Steps in Detail

Before You Start

- Dissolve lyophilized **Proteinase K** in 2,5 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 miRNA 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. Pumps 1, 5 and 6 are not used with this kit.

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

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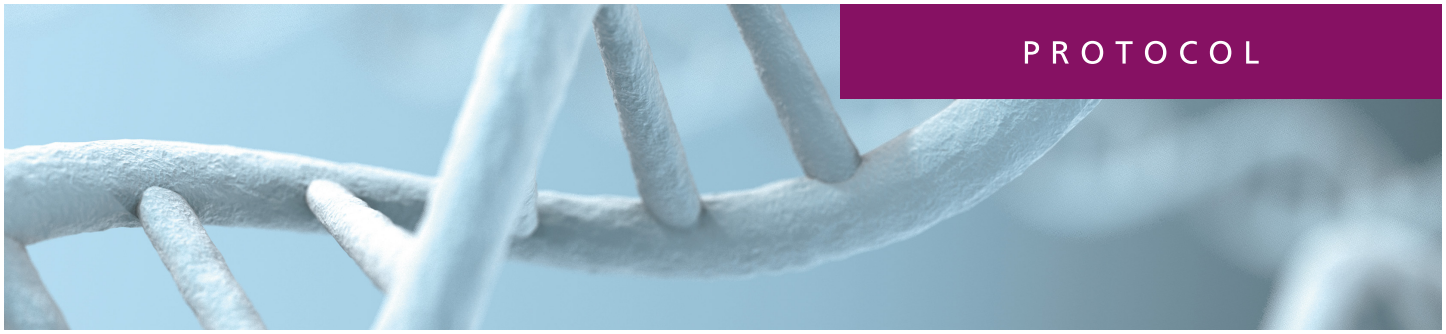

Protocol Steps

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 "**chemagic miRNA 200 H96 drying prefilling VD210726**" 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** according to the sample positions.

As a general rule, 10 - 20 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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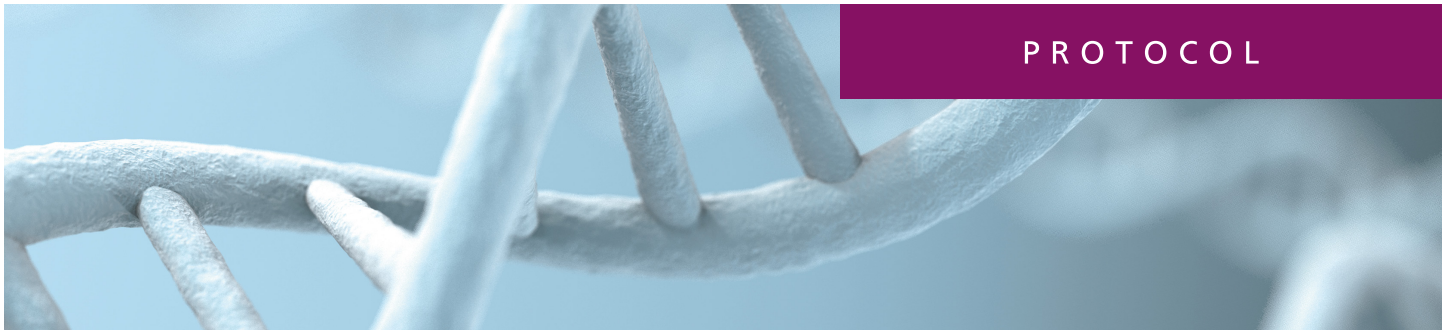

9. Prefill the selected wells of the Sample Plate with 200 µL plasma equilibrated to room temperature. Add 25 µL **Proteinase K** and 100 µL **Lysis Buffer 1.**

   Proteinase K and **Lysis Buffer 1** can be premixed (choose the appropriate volume of **Proteinase K** / **Lysis Buffer 1** to ensure you have a sufficient amount 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 / 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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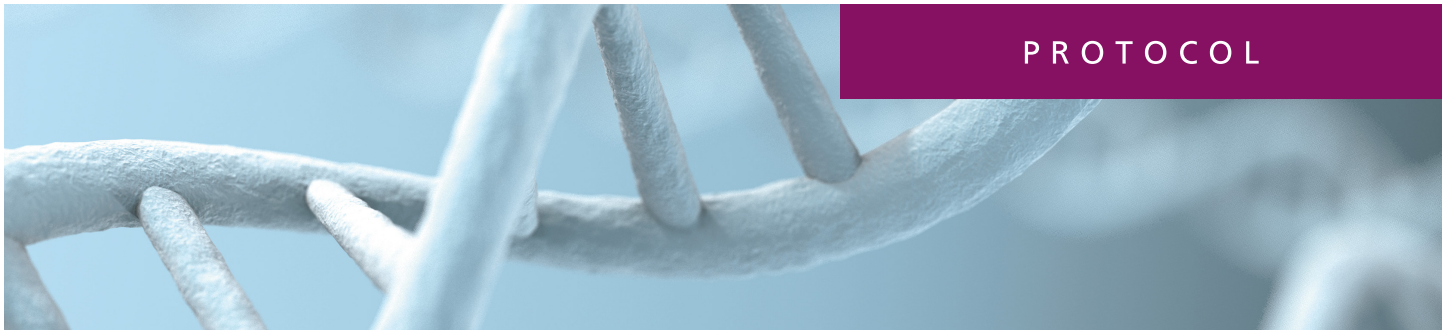

Cleaning Information

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.

Any further questions?

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