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Purification Protocol for Viral DNA/RNA from 300 µl Plasma, Serum, Naso- or Oropharyngeal Swabs, Saliva, BAL and Sputum Using the chemagic 360 with integrated chemagic Dispenser

Protocol name: **chemagic Viral300 360 H96 drying prefilling VD200309.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 150 µl **Magnetic Beads**

Position 3: deep-well-plate (riplate SW) containing
- 300 µl sample
- 4 µl **Poly(A) RNA**
- 10 µl **Proteinase K**
- 300 µl **Lysis Buffer 1**
- **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 5** added automatically]

Position 7: deep-well-plate (riplate SW) prefilled with 50 - 100 µl **Elution Buffer 6**

Any further questions?

chemagen Technology technical support: +49 (0) 2401 805-501 | support.chemagen@perkinelmer.com

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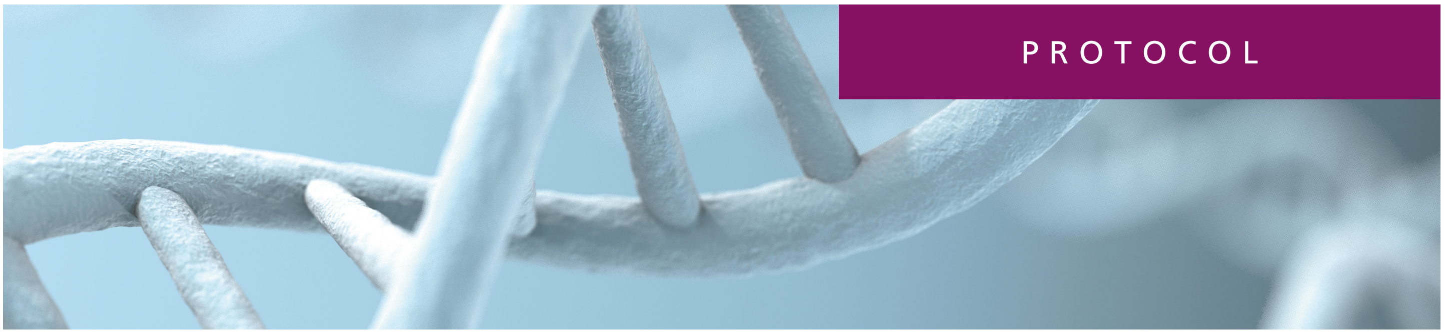

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Sample Material

The **chemagic Viral DNA/RNA Kit** can be used for plasma and serum, but also for different kinds of respiratory swabs and samples. Plasma, serum, saliva and transport media from naso- or oropharyngeal swabs can be used directly in aliquots of 300 µl per isolation. Sample material from dried swabs, bronchoalveolar lavage (BAL) and sputum has to be liquefied before use.

Processing Steps in Detail

Before You Start

- Dissolve lyophilized **Poly(A) RNA** by adding 440 µl of the **Poly(A) RNA Buffer** to the **Poly(A) RNA** tube and mix thoroughly.
- Dissolve lyophilized **Proteinase K** in H_2O (volume is given on the label).

! ***See below "Storage Conditions".***

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 Viral300 360 H96 drying prefilling VD200309.che**" and press the [**Insert IDs**] button. Follow the instructions as given in the 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 supply bottles contain enough buffer. Only if the liquid level for all buffers is above 125 ml 96 isolations can be performed. Otherwise please fill up the buffer supply containers or exchange against a new one.***

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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 selected wells of the Sample Plate with 300 µl sample.
7. Prefill the **Elution Buffer 6** and the thoroughly resuspended **Magnetic Beads** according to the sample positions.

! ***For indication of volumes and sample positions see “Positioning Tips and Plates on the Tracking System”.***

8. Add 4 µl **Poly(A) RNA**, 10 µl **Proteinase K** and then 300 µl **Lysis Buffer 1** to the wells containing sample.

   It is possible to premix **Poly(A) RNA**, **Proteinase K** and **Lysis Buffer 1** (choose the appropriate volume of **Poly(A) RNA** / **Proteinase K** / **Lysis Buffer 1** to ensure you have sufficient 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 Poly(A) RNA / Proteinase K /* Lysis Buffer 1 *within this time.***

9. Place the plates on the tracking system according to the instructions given by the **chemagic QA software**.
10. Place the Sample Plate in **position 3** on the tracking system.
11. Check all plates for accurate orientation and fitting.
12. Close the 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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PROTOCOL

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Additional Information

Safety Information

Always wear a laboratory coat, disposable gloves and protective goggles. For detailed information please consult the appropriate material safety data sheet (SDS).

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 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.che**” instead of the regular one for this purpose.

! ***If the chemagic Dispenser will not be used for longer period of 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.

Storage Conditions

All **Wash Buffers**, the **Binding Buffer 2** and the **Magnetic Beads** can be stored at room temperature.

Store **Lysis Buffer 1** and **Poly(A) RNA Buffer** in the dark.

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

The reconstituted **Poly(A) RNA** in **Poly(A) RNA Buffer** is stable for 4 weeks at 4 °C and the reconstituted **Proteinase K** is stable for 2 weeks at 4 °C.

For long term storage we recommend to store the reconstituted **Poly(A) RNA** and **Proteinase K** in aliquots at -20 °C. Do not freeze the **Poly(A) RNA** and **Proteinase K** aliquots after thawing.

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.

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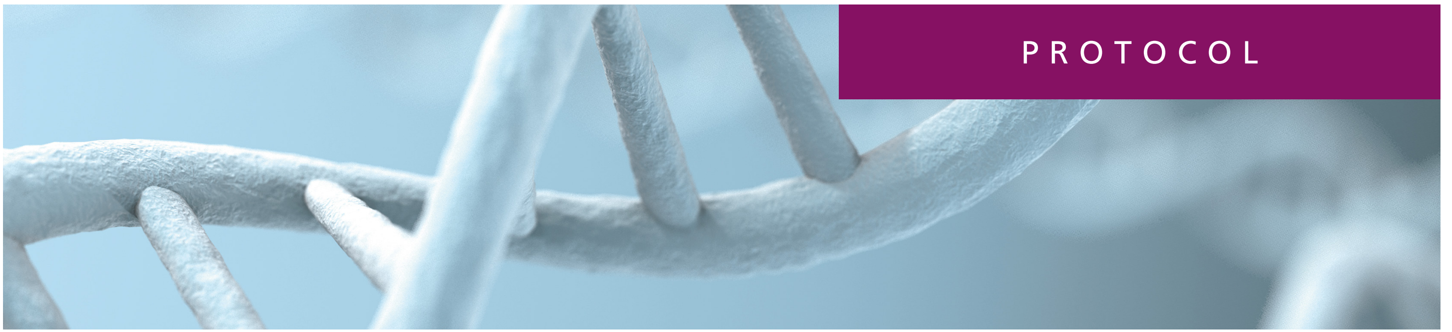

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General Remarks

Deep-well-plates (riplate SW) and the low-well-plates (MICROTITER SYSTEM) are delivered from chemagen optionally. You can use your own plates also, but the instrument protocol has to be adapted to the specific plates.

The **Elution Buffer 6** 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.

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 to perform an additional separation step using an appropriate **chemagic** magnetic stand in order to separate traces of particles.

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