Prepito Viral DNA/RNA D200 Kit

(art. No. D-2015)

CE

PerkinElmer®

Symbols

	Kit contains reagents for 180 preparations
	Refer to information given in the handbook V120328
	Expiry date
	Lot number
	in vitro diagnostic medical device
	Temperature limitations
	D-2015
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Intended Use

With the **Prepito Viral DNA/RNA D200 Kit** viral nucleic acids can be isolated from plasma or serum for subsequent in vitro diagnostic purposes. The Kit has to be used with the **chemagic Prepito**.

The product is intended for professional users such as technicians and physicians trained in molecular biology techniques. To minimize irregularities in diagnostic results, the product should always be used with an internal control as well as positive and negative controls throughout the process of sample preparation, sample amplification and detection according to the downstream assay used..

Any diagnostic results generated using the sample preparation procedure in conjunction with any downstream diagnostic NAT assay should be interpreted with regard to other clinical or laboratory findings.

Content

Functional principle

The chemagic **Prepito Viral DNA/RNA D200 Kit** is based on chemagen's proprietary magnetic bead technology platform. Viruses in the sample material are lysed during the isolation process. The released nucleic acids bind to small magnetisable particles which are then magnetically separated from the sample material. During subsequent steps contaminations are removed and the purified nucleic acids are transferred into an elution medium. The automated sample processing by the **chemagic Prepito** excludes cross contamination and ensures a safe handling of infectious sample material..

Quality control

Each lot is tested for its defined specifications according to chemagen's Quality Management System. Procedures that are not in accordance with this manual could cause inadequate results.

Product limitations

The Kit is designed for the use with humane plasma or serum. The Kit is not intended for the use with tissue or blood sample material. The isolation efficiency with other types of sample material has not been determined.

Stability and storage

Expiry dates are stated on the box and the single components of the kit. Do not use any components of the kit beyond the expiry date. All kit components can be stored at room temperature.

Lysis Buffer 1 (plasma) and Poly(A) RNA Buffer have to be stored in the dark. Lysis Buffer 1 may form a precipitate upon storage. If necessary, warm to approximately 55 °C to redissolve. Precipitates in the Poly(A) RNA buffer can be redissolved at room temperature.

After reconstitution Protease solution and Poly(A) RNA solution have to be stored at 4 °C. The solutio ns can be used for 6 weeks. For long term storage we recommend aliquoting the Proteinase K solution and the Poly(A) RNA solution and storing at –20 °C.

Protocol duration

The length of the purification protocol is 70 min.

Contents of the Kit unit (corresp. to 180 preparations from 200 µL serum or plasma)

1. **Magnetic Beads** 30 mL
2. **Lysis Buffer 1**: 45 mL

   Guanidine thiocyanate 43 – 50 %
3. **Binding Buffer 2***: 130 mL

   Tris-HCl-buffer,
   Sodium perchlorate 25 – 28 %,
   Ethanol 45 – 60 %
4. **Wash Buffer 3***: 110 mL

   Tris-HCl-Puffer,
   Sodium perchlorate 15 – 18 %,
   Ethanol 20 – 25 %
5. **Wash Buffer 4***: 110 mL

   Ethanol 70 – 80 %
6. **Elution Buffer 5**: 30 mL

   10 mM; Tris-HCl-buffer pH 8.0
7. **Protease**: 2.0 mL
8. **Poly(A) RNA**: 2 x 350 µg
9. **Poly(A) RNA Buffer**: 2 x 440 µL
10. **Disposable Tips**: 180
11. **2 mL Deep Well Plates**: 15
12. **0.75 mL Reaction Tubes**: 360
13. **0.75 mL Caps**: 180

*included in the **chemagic 8-Pack**

Safety

To avoid injuries while working with the kit components, always wear safety glasses, disposable gloves, and protective clothing. For detailed information, please refer to the according material safety data sheet (MSDS).

Reagent 1: Magnetic Beads, no hazardous ingredients

Reagent 2: Lysis Buffer 1

Guanidine thiocyanate, CAS No. 593-84-0, EC No. 209-812-1,
Xn R20/21/22-32-52/53, S13-61

Reagent 3: Binding Buffer 2

Sodium perchlorate CAS No.7601-89-0 EC No.231-511-9, Xn R9-22, S13-22-27
Ethanol CAS No.64-17-5 EC No.200-578-6, F R11

Reagent 4: Wash Buffer 3

Ethanol CAS No.64-17-5 EC No.200-578-6, F R11, S7-16
Sodium perchlorate CAS No.7601-89-0 EC No.231-511-9, Xn R9-22, S13-22-27

Reagent 5: Wash Buffer 4

Ethanol CAS No.64-17-5 EC No.200-578-6, F R11, S7-16

Reagent 6: Elution Buffer 5, no hazardous ingredients

Reagent 7: Protease

Protease CAS No.9036-06-0 EC No.232-909-5, Xn R36/37/38-42/43 S22-24-26
36/37/39 46

Reagent 8: Poly(A) RNA, no hazardous ingredients

Reagent 9: Poly(A) RNA Buffer

Guanidine thiocyanate, CAS No.593-84-0, EC No.209-812-1,
Xn R20/21/22-32-52/53, S13-61

Equipment and other material to be provided by the user

chemagic Prepito, RNAse-free water, disposable gloves, pipette and pipette tips with aerosol barrier (ensure that all used material is RNase free).

Purification Protocol using the chemagic Prepito

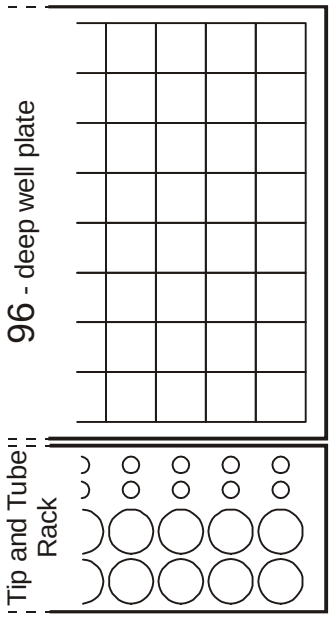
The protocol is suitable for up to 12 samples in parallel (see protocol steps below). (Detailed instructions for the use of the **chemagic Prepito** can be found in the corresponding user manual).

Before you start:

- Check all kit components for integrity. In case of damages contact your supplier.
- Connect the tubes according to their numbering to the respective counterparts at the **chemagic 8-Pack**. Remove the lids from the individual buffer bottles in the **chemagic 8-Pack** and pierce the septum with the spike at the end of the tube. Place the **chemagic 8-Pack** upside down on the reagent holder.
- Dissolve the lyophilized **Protease** in RNAse-free water (see instruction on the tube) and **Poly(A) RNA** in 440 µL **Poly(A) RNA Buffer** per tube.

Positioning of the Deep Well Plate and the chemagic Tip & Tube Rack

The following scheme shows the orientation of the 96 Deep Well Plate and the **chemagic Tip & Tube Rack**. For detailed information see protocols steps.



200 µL sample material, Protease and Poly(A)RNA)

Pos. 4 second row for Disposable Tips; **! not used in this protocol !**
Pos. 3 Disposable Tips

Pos. 2 0.75 mL reaction tubes with 150 µL **Magnetic Beads**

Pos. 1 0.75 mL reaction tubes with 50 - 100 µL **Elution Buffer**

Protocol Steps (chemagic Prepito serial numbers 1 – 99)

1. Switch on the **chemagic Prepito** and wait for the self test to finish.
2. Press [**change protocol**].
3. Select the **Prepito Viral DNA/RNA D200 Kit** protocol by pressing [**Viral DNA/RNA 200**].
4. Enter the access code [**2365**] for authorization and confirm by pressing **[enter]**.
5. Confirm the selection of the correct protocol by pressing [**enter**].
6. Read the protocol information in the appearing information screen. Confirm by pressing [**continue**].
7. Select the sample positions and confirm by pressing [**continue**].
8. Enter the kit barcode with the barcode scanner and confirm by pressing [**ok**].
9. For the registration of the samples and storage tubes press [**yes**] and follow the instructions on the touch screen panel to enter the according barcodes.
10. Prepare the **chemagic Tip & Tube Rack** with the required materials. Place one 0.75 mL reaction tube filled with 50 - 100 µL **Elution Buffer** (position 1), one 0.75 mL reaction tube filled with 150 µL of **Magnetic Beads** (position 2) and one disposable tip (position 3) for each sample into positions according to the sample positions.

    Shake the Magnetic Bead solution vigorously until all Magnetic Beads are completely suspended. An incomplete resuspension of the Magnetic Bead solution could cause a decreased yield of extracted nucleic acids.

11. Add 4 µL **Poly(A) RNA** solution and 10 µL **Protease** into the sample position of the Deep Well Plate (DWP, riplate SW).
12. Add 200 µL sample material and 200 µL **Lysis Buffer 1** into the well prefilled with **Poly(A) RNA** and **Protease** solutions.
13. An information screen indicates the previously selected sample positions. Ensure that the sample positions in the DWP correspond to the selected positions. Place the DWP on its default position on the tracking system and press [**continue**].
14. Place the **chemagic Tip & Tube Rack** on its default position on the tracking system. Check the accurate fit of the DWP and **chemagic Tip & Tube Rack** and lock both by closing the safety latch.
15. Close the front door and immediately start the automated isolation process by pressing [**start**].

Protocol Steps (chemagic Prepito serial numbers 100 and later)

1. Switch on the **chemagic Prepito** and wait until the self test is finished.
2. Press [**Change Protocol**].
3. Press [**Serum/Plasma**] in the Select Protocol Group window
4. Select the **Prepito Viral DNA/RNA200 Kit Kit** protocol by pressing [**Viral DNA/RNA 200**] and confirm by pressing [**OK**].
5. Confirm the protocol selection in the Select Protocol Group window by pressing [**OK**]
6. Enter the 4 digit access code [**2365**] for authorization and confirm by pressing [**Enter**].
7. Press [**Start Process**].
8. Read the protocol information in the appearing information screen and confirm by pressing [**Continue**].
9. Select the sample positions and confirm by pressing [**OK**].
10. Enter the kit barcode with the barcode scanner and confirm by pressing [**OK**].
11. For the registration of the samples and elution tubes press [**Yes**] and follow the instructions on the touch screen panel to enter the according barcodes.
12. Prepare the **chemagic Tip & Tube Rack** with the required material. Place one 0.75 mL reaction tube filled with 50 - 100 µL **Elution Buffer** (position 1), one 0.75 mL reaction tube filled with 150 µL **Magnetic Beads** (position 2) and one **Disposable Tip** (position 3) for each sample into the positions according to the sample positions.

    Shake the vessel with the Magnetic Beads vigorously until all Magnetic Beads are completely resuspended. An incomplete resuspension of the Magnetic Beads can result in a decreased yield of extracted nucleic acids.

13. Add 4 µL **Poly(A) RNA** solution and 10 µL **Protease** solution into the sample position of the Deep Well (DWP) defined as sample wells (Pos. “lysate mix”, see section above “Positioning Procedure”).
14. Add 200 µL sample material and 200 µL **Lysis Buffer** into the well filled with **Poly(A) RNA** and **Protease** solutions.
15. An information screen indicates the previously selected sample positions. Ensure that the sample positions in the DWP correspond to the selected positions. Place the DWP on its default position on the tracking system and press [**Continue**].
16. Place the **chemagic Tip & Tube Rack** on its default position on the tracking system. Check for accurate fit of the DWP and the **chemagic Tip & Tube Rack** and lock both by closing the safety latch.
17. Close the front door and immediately start the automated isolation process by pressing [**Start**].

General remarks

It is strongly recommended to use the extracted nucleic acids immediately for amplification. If nucleic acid extracts cannot be used for amplification directly after preparation, the nucleic acid extracts can be kept at -20 ℃ or preferably at -70 ℃ for up to one month or one year respectively.

The **Elution Buffer** included in this kit is 10 mM Tris-HCl pH 8.0.

Troubleshooting

Problem	Possible Cause	Recommendation/Solution
Red eluates/low detection sensitivity	Traces of erythrocytes in the plasma or serum samples	Avoid to carry over erythrocytes during the preparation of plasma or serum
Low detection sensitivity for positive controls and/or target nucleic acid	Incorrect amount of **Magnetic Beads** added	Resuspend the **Magnetic Beads** well before adding to the lysate
	Insufficient lysis	Add the correct volume of lysis buffer
	Buffers in the **chemagic 8-Pack** are not connected to the machine	*Connect the buffers in the **chemagic 8-Pack** to the machine
	The **chemagic 8-Pack** is not positioned in the right manner on the reagent holder	*Place the **chemagic 8-Pack** in the correct position on the reagent holder
	Tubes contain air after connecting the **chemagic 8-Pack** to the machine	*Fill the tubes completely using the manual priming function
	Buffers in the **chemagic 8-Pack** are empty	*Change the **chemagic 8-Pack.** Don't use the **chemagic 8-Pack** for more than the indicated preparations
	Buffers of the **chemagic 8-Pack** are not connected in the right manner to the **chemagic Prepito**	*Check/correct the connections between the **chemagic Prepito** and the **chemagic 8-Pack**
	Irregular dispensing of the buffers	*Check the calibration of the pumps
Contaminated or inactive Protease	Visible microbial growth in **Protease** solution	Use sterile water for resuspension of the **Protease**
	Incorrect storage of the **Protease** solutions	Store **Protease** solution at 4 °C; do not use the solutions longer than 6 weeks Store aliquots at –20 °C Avoid thawing-freezing cycles
Malfunction of the instrument	e.g. mechanical, electrical or electronical problems	Contact chemagen or your local supplier

*detailed information is given in the manual of the **chemagic Prepito**