PROTOCOL

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

# Prepito Viral DNA/RNA1k Kit

*Simultaneous isolation of viral DNA and RNA from 1000 µL serum or plasma*

## Kit Components

| | |
|---|---|
| **Magnetic Beads** | **Poly(A) RNA** |
| **Lysis Buffer** | **Poly(A) RNA Buffer** |
| **Binding Buffer** | **Proteinase K** |
| **Wash Buffer 3** | **Deep Well Plates** |
| **Wash Buffer 4** | **0.75 µL Reaction Tubes** |
| **Elution Buffer** | **Disposable Tips** |

**Completion time:** approximately 90 minutes

## Storage Conditions and Safety Information

Expiry dates are stated on the box and on each single component of the kit. Do not use any component of the kit beyond the expiration date. All kit components can be stored at room temperature. The kit buffers contain irritant substances. Take appropriate laboratory safety measures and wear gloves when handling. The included protocol is sufficient for most plasma and serum samples: fresh, non-coagulated and frozen. This kit is optimized for DNA/RNA purification from human serum samples.

**Lysis Buffer** and **Poly(A) RNA Buffer** have to be stored in the dark. Lysis Buffer may form a precipitate upon storage. If necessary, warm to 55 °C to redissolve (after heating the **Lysis Buffer** can



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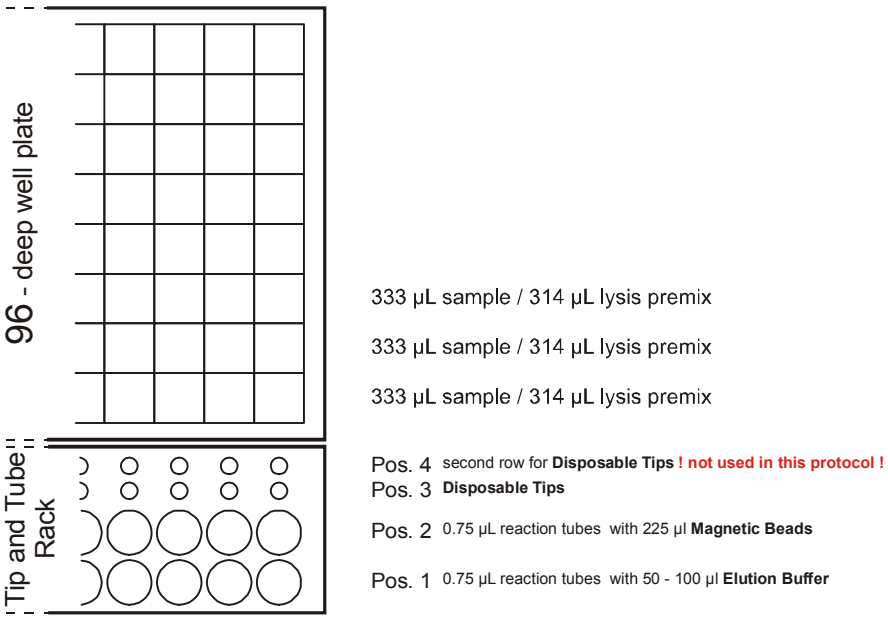
directly be used in the isolation process without the need of cooling down). Precipitates in the **Poly(A) RNA Buffer** can be redissolved at room temperature.

After dissolving **Proteinase K** solution and **Poly(A) RNA** solution have to be stored at 2 – 8 °C. The solutions 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.

## Positioning Procedure

*See "**Protocol Steps**"for detailed Information.*



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## 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 placed at the end of each tube. Place the **chemagic 8-Pack** upside down on the reagent holder and use the manual priming function for a complete filling of the dispensing system.
- Dissolve the lyophilized **Proteinase K** in RNase-free water (see instruction on the tube) and **Poly(A) RNA** in 440 µL **Poly(A) RNA Buffer** per tube.



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## Protocol Steps (chemagic Prepito serial numbers 1 – 99)

1. Switch on the **chemagic Prepito** and wait until the self test is finished.
2. Press [**change protocol**].
3. Select the **Prepito Viral DNA/RNA 1k** **protocol by pressing** [**Viral DNA/RNA200**].
4. Press [**continue**].
5. Enter the access code [**3117**] for authorization and confirm by pressing [**enter**].
6. Confirm the selection of the correct protocol by pressing [**enter**].
7. Read the protocol information in the appearing information screen. Confirm by pressing [**continue**].
8. Select the sample positions and confirm by pressing [**continue**].
9. Enter the kit barcode with the barcode scanner and confirm by pressing [**ok**].
10. 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.
11. 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 225 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.***

12. Of the 1 mL sample, add 3 x 333 µL serum to the first three cavities of the Deep Well Plate (DWP) defined as sample well (Pos. “333 µL sample/314 µL lysis premix”, see section above “Positioning Procedure”).



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13. Prepare a lysis premix containing 12 µl **Poly(A) RNA** solution, 30 µL **Proteinase K** and 900 µL **Lysis Buffer** for each sample.

! ***The activity of Proteinase K will be decreased by the incubation in Lysis Buffer. Ensure not to exceed an incubation time longer than 10 minutes prior to the addition to the samples.***

14. Add 314 µL lysis premix to each sample position.
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**].

## 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/RNA1k Kit** protocol by pressing [**Viral DNA/RNA1k**] 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 [**3117**] for authorization and confirm by pressing [**Enter**].
7. Press [**Start Process**].



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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 225 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. Of the 1 mL sample add 3 x 333 µL serum to the first three cavities of the Deep Well Plate (DWP) defined as sample well (Pos. “333 µL sample/314 µL lysis premix”, see section above “Positioning Procedure”).
14. Prepare a lysis premix containing 12 µl **Poly(A) RNA** solution, 30 µL **Proteinase K** and 900 µL **Lysis Buffer** for each sample.

! ***The activity of Proteinase K will be decreased by the incubation in Lysis Buffer. Ensure not to exceed an incubation time longer than 10 minutes prior to the addition to the samples.***

15. Add 314 µL lysis premix to each sample position.
16. 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**].



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17. 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.
18. Close the front door and immediately start the automated isolation process by pressing [**Start**].

## General Remarks

The **Elution Buffer** 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. RNase free 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/RNA yield could be reduced.



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