

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

## Prepito Circulating NA1k Kit

Isolation of circulating DNA and RNA from 1 ml serum or plasma

### Kit Components

<b>Magnetic Beads</b>	<b>Poly(A) RNA</b>
<b>Lysis Buffer</b>	<b>Poly(A) RNA Buffer</b>
<b>Binding Buffer</b>	<b>Proteinase K</b>
<b>Wash Buffer 3</b>	<b>Deep Well Plates</b>
<b>Wash Buffer 4</b>	<b>0.75 mL Reaction Tubes</b>
<b>Elution Buffer</b>	<b>Disposable Tips</b>

**Completion time:** approximately 140 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 expiry 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.

**Poly(A) RNA Buffer** have to be stored in the dark. **Poly(A) RNA Buffer** may form a precipitate upon storage, which can be dissolved 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 4 weeks. For long term storage we recommend aliquoting the **Proteinase K** solution and the **Poly(A) RNA** solution and storing at – 20 °C.

Any further questions?

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

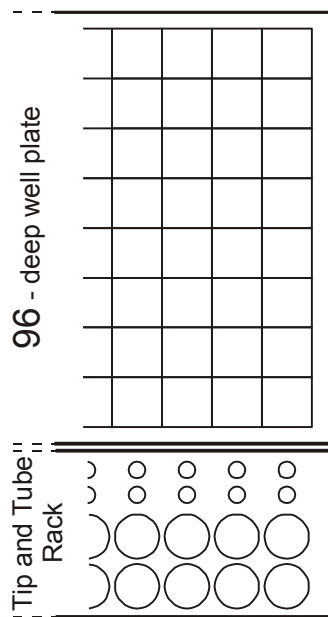




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## Positioning Procedure

See "Protocol Steps" for detailed Information.



50  $\mu$ L **Magnetic Beads**

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650  $\mu$ L lysate (sample/**Lysis Buffer**)

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

Pos. 3 **Disposable Tips**

Pos. 2 empty

Pos. 1 0.75 mL reaction tubes with 60 - 100  $\mu$ L **Elution Buffer**



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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  $\mu$ L **Poly(A) RNA Buffer** per tube.

## Equipment and Other Material to be Provided by the User

RNase-free water, disposable gloves, pipette and pipette tips with aerosol barrier, 2 mL reaction tubes, and heat incubator or a water bath (ensure that all used material is RNase free).

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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 Circulating NA1k** protocol by pressing [**Viral DNA/RNA200**].
  4. Press [**continue**].
  5. Enter the access code [**3225**] 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 60 - 100  $\mu$ L **Elution Buffer** (position 1) and one **Disposable Tip** (position 3) for each sample into the positions according to the sample positions.
  12. Pipette 1 mL sample material into a 2 mL reaction tube.
  13. Prepare a lysis premix containing 7  $\mu$ L **Poly(A) RNA** solution, 30  $\mu$ L **Proteinase K** and 900  $\mu$ L **Lysis Buffer** for each sample. Ensure that the amount of lysis premix is sufficient for the number of samples have to be prepared.
- !** *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 937  $\mu$ L lysis premix to each sample and incubate for 60 minutes at 60 °C.
  15. Aliquot the **Magnetic Beads** by adding 3 x 50  $\mu$ L to the cavities 4-6 of the Deep Well Plate (DWP) defined as Magnetic Bead wells (Pos. "Magnetic Beads", see section above "Positioning Procedure").

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**!** *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.*

16. Aliquot the lysate (sample/lysis premix) by adding 3 x 630 µL to the first three cavities of the Deep Well Plate (DWP) defined as sample wells (Pos. "lysate (sample/lysis premix)", see section above "Positioning Procedure").
17. 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].
18. 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.
19. 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 Circulating NA1k Kit** protocol by pressing [Circulating NA1k] 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 [3225] 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].

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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 60 -100  $\mu$ L **Elution Buffer** (position 1) and one **Disposable Tip** (position 3) for each sample into the positions according to the sample positions.
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**!** *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.*

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

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## 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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