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Support protocol: Isolation of viral RNA and DNA from cell-free biological fluids with NucleoSpin® RNA Virus F

The standard protocol is recommended for the purification of viral RNA and viral DNA for all types of DNA viruses like HBV and CMV.

Before starting the preparation:

Check if Wash Buffer RAV3 was prepared according to section 3. Preheat an aliquot of Elution Buffer RE / RNase-free H₂O to 70°C.

1) Lysis of viruses

Add 4 ml Buffer RAV1 containing Carrier RNA to 1 ml of the fluid sample.

Add $\underline{130~\mu l}$ Proteinase K (20 mg / ml stock solution) to the lysis mixture. Pipette mixture up and down and vortex for 10 - 15 s.

Incubate for 5 min at 70°C.

Incubation time and temperature are critical for lysis as well as RNA stability (see troubleshooting of the NucleoSpin® RNA Virus F User Manual).

Proteinase K is not included in the NucleoSpin® RNA Virus F kit, but can be ordered separately (Cat. No. 740506: 100 mg Proteinase K).

If the resulting solution is still turbid, centrifuge the mixture for 1 min at $11,000 \times g$ to pellet particles, to prevent clogging of the NucleoSpin[®] RNA Virus F Columns.

Take off the supernatant and <u>continue with step 2 of protocol 5.3</u> of the User manual.