Contents

Recommended Starting Material

The size of recommended starting material to use with determined lysis volume are listed here.

Table: Recommended starting material and Lysis Buffer amount

Sample	Size of Starting material	Lysis Buffer Amount
Plasma/serum	Up to 200 µl	200 µl

Protocol

Isolation of Viral DNA (based on silica technology)

Sample type:

- Body fluid
- Serum
- Plasma

Some tips to know

- All centrifugation steps are carried out at room temperature (15–25°C).
- Equilibrate samples to room temperature.
- Equilibrate ERR to room temperature for elution step.
- Prepare a 56°C thermoblock for use.
- Add carrier RNA reconstituted in ERR to GLB according to following instructions.
- Add ethanol (96–100%) to the bottle containing BWB1[1] concentrate, as described on the bottle. Store reconstituted BWB1 at room temperature (15–25°C).

Note: Always mix reconstituted Buffer BWB1 by shaking before starting the procedure.

- Add ethanol (96–100%) to the bottle containing BWB2 concentrate, as described on the bottle. Store reconstituted BWB2 at room temperature (15–25°C).

[1] Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach

Note: Always mix reconstituted BWB2 by shaking before starting the procedure.

- If GL Buffer (GLB) forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Addition of carrier RNA to GLB[2]

Carrier preparation

Add 620 µl ERR to the tube containing 620 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl (for 5 Prep sample add 31 µl ERR to the tube containing 31 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl). Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at –20°C. Do not freeze–thaw the aliquots of carrier RNA more than 3 times.

Important! Note that carrier RNA does not dissolve in GLB. It must first be dissolved in ERR and then added to GLB.

GLB preparation

Calculate the volume of Buffer GLB–carrier RNA mix needed per batch of samples to be simultaneously processed from Table 1.

Attention! Mix gently by inverting the tube 10 times. To avoid foaming, do not vortex.
Note: The sample-preparation procedure is optimized for 5.6 µg of carrier RNA per sample. If less carrier RNA has been shown to be better for your amplification system, transfer only the required amount of dissolved carrier RNA to the tubes containing GLB.

[2] Contains chaotropic salt. Take appropriate laboratory safety measures and wear gloves when handling. Not compatible with disinfectants containing bleach.

For each microgram of carrier RNA required per preparation, add 5 µl ERR-dissolved carrier RNA per milliliter of GLB. (Using less than 5.6 µg carrier RNA per sample must be validated for each particular sample type and downstream assay.)

Table 1. Volumes of GLB and carrier RNA–ERR mix required for the procedure

Samples Number	GLB amount (ml)	Carrier RNA–ERR amount(µl)	Samples Number	GLB amount (ml)	Carrier RNA–ERR amount (µl)
1	0.22	6.2	13	2.86	80.1
2	0.44	12.3	14	3.08	86.3
3	0.66	18.5	15	3.30	92.4
4	0.88	24.6	16	3.52	98.6
5	1.10	30.8	17	3.74	104.7
6	1.32	37.0	18	3.96	110.9
7	1.54	43.1	19	4.18	117.0
8	1.76	49.3	20	4.40	123.2
9	1.98	55.4	21	4.62	129.4
10	2.20	61.6	22	4.84	135.5
11	2.42	67.8	23	5.06	141.7
12	2.64	73.9	24	5.28	147.8

Process

1. Pipet 25 µl RJ-Protease into a 1.5 ml clean microcentrifuge tube. Add 200 µl of sample (plasma, serum, body fluid etc.) into the tube.

Note: If the sample volume is less than 200 µl, add the appropriate volume of 0.9% sodium chloride solution to bring the total volume up to 225 µl (including RJ-Protease).

2. Add 200 µl GLB (containing 28 µg/ml of carrier RNA). Mix by pulse vortexing for 15 s.

Note: It is crucial that the sample and GLB are mixed thoroughly to yield a homogeneous solution.

Note: Do not add RJ-Protease directly to GLB.

3. Incubate at 56 °C for 15 min in a thermoblock. Then, centrifuge briefly to remove drops from the inside of the lid.
4. Add 250 µl of ethanol (96–100%) to the sample; mix thoroughly by pulse vortexing for 15 s. Incubate the lysate with the ethanol for 5 min at room temperature (15–25°C).

Note: Cool ethanol on ice before adding to the lysate, if ambient temperature exceeds 25 °C.

5. Centrifuge the tube briefly to remove drops from the inside of the lid. Carefully pipette all of the lysate from previous step onto the HiPure VI column without wetting the rim.
6. Centrifuge at 6000 x g (8000 rpm) for 1 min. Discard the flow-through and place back the HiPure DR column in the collection tube.

Note: If the lysate did not pass the column, centrifuge at full speed until it passes through the column.

7. Add 500 µl BWB1 and centrifuge at 6000 x g (8000 rpm) for 1 min. Discard the flow-through and place back the HiPure DR column in the collection tube.

Note: This step increases kit performance when processing inhibitory samples.

8. Add 500 µl BWB2 and centrifuge at 6000 x g (8000 rpm) for 1 min. Discard the flow-through and place back the HiPure DR column in the collection tube.
9. Add 500 µl ethanol (96–100%) and centrifuge at 6000 x g (8000 rpm) for 1 min. Discard the flow-through.

Attention! Ethanol carryover into the eluate may cause problems in downstream applications. Removing the HiPure VI column and collection tube from the rotor may also cause flow-through to come into contact with the HiPure VI column.

10. Place back the HiPure VI column in the collection tube. Centrifuge at full speed 20000 x g (14000 rpm) for 3 min to dry the membrane completely. Then discard both the collection tube and flow-through.

Recommended: Place the HiPure VI column into a new 2 ml collection tube (not provided), open the lid, and incubate the assembly at 56 °C for 3 min to dry the membrane completely.

*This step serves to evaporate any remaining liquid.

11. Place the HiPure VI column in a clean 1.5 ml clean microcentrifuge tube (not provided). Apply 20–150 µl ERR to the center of the membrane. Close the lid and incubate at room temperature for 5 min.
12. Centrifuge at full speed, 20000 x g (14000 rpm) for 1 min.

* Ensure that ERR is equilibrated to room temperature.

Important! If elution is done in small volumes (<50 µl), ERR must be dispensed onto the center of the membrane for complete elution of bound RNA and DNA.

Note: Incubation after adding ERR for 5 min at room temperature before centrifugation generally increases DNA and RNA yield.