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 BFC according to following instructions.
- Add ethanol (96–100%) to a 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 a bottle containing Buffer BWB2 concentrate, as described on the bottle. Tick the check box on the label to indicate that

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

ethanol has been added. Store reconstituted BWB2 at room temperature (15–25°C).

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

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

Addition of carrier RNA to BFC[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 BFC. It must first be dissolved in ERR and then added to BFC.

BFC preparation

Calculate the volume of Buffer BFC–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.

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

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 BFC. For each microgram of carrier RNA required per preparation, add 5 µl ERR-dissolved carrier RNA per milliliter of BFC. (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 BFC and carrier RNA–ERR mix required for the procedure

Samples Number	BFC amount (ml)	Carrier RNA–ERR amount(µl)	Samples Number	BFC amount (ml)	Carrier RNA–ERR amount (µl)
1	0.56	5.6	13	7.28	72.8
2	1.12	11.2	14	7.84	78.4
3	1.68	16.8	15	8.4	84.0
4	2.24	22.4	16	8.96	89.6
5	2.80	28.0	17	9.52	95.2
6	3.36	33.6	18	10.08	100.8
7	3.92	39.2	19	10.64	106.4
8	4.48	44.8	20	11.20	112.0
9	5.04	50.4	21	11.76	117.6
10	5.60	56.0	22	12.32	123.2
11	6.16	61.6	23	12.88	128.8
12	6.72	67.2	24	13.44	134.4

Process

1. Pipet 560 µl prepared BFC containing carrier RNA into a 1.5 ml clean microcentrifuge tube. Add 140 µl plasma, serum, urine, cell-culture supernatant or cell-free body fluid to the Buffer BFC–carrier RNA in the microcentrifuge tube. Mix by pulse vortexing for 15 s.

   Note: If the sample volume is larger than 140 µl, increase the amount of Buffer BFC–carrier RNA proportionally (for example, a 280 µl sample will require 1120 µl Buffer BFC–carrier RNA) and use a larger tube.

   Note: It is important that the sample is mixed thoroughly with BFC to yield a homogeneous solution. Frozen samples that have only been thawed once can also be used.

2. Incubate at room temperature (15–25°C) for 10 min.

   Attention! Viral particle lysis is complete after lysis for 10 min at room temperature. Longer incubation times have no effect on the yield or quality of the purified RNA.

3. Centrifuge briefly the tube to remove drops from the inside of the lid. Add 560 µl ethanol (96–100%) to the sample, and mix by pulse vortexing for 15 s. Then, centrifuge the tube to remove drops from inside the lid. To ensure efficient binding, it is essential that the sample is mixed thoroughly with the ethanol to yield a homogeneous solution.

   Attention! Use only ethanol, since other alcohols may result in reduced RNA yield and purity. Do not use denatured alcohol, which contains other substances such as methanol.

Note: If the sample volume is greater than 140 µl, increase the amount of ethanol proportionally (for example a 280 µl sample will require 1120 µl ethanol).

4. Gently, pipette 630 µl of the mixture to a HiPure DR column placed in a 2 ml collection tube, without wetting the rim (supplied in the kit box). Centrifuge at 6000 x g (8000 rpm) for 1 min. Discard flow-through and place back the HiPure DR column in to the collection tube.

   Note: Close each spin column to avoid cross-contamination during centrifugation.

   Note: If the solution has not completely passed through the membrane, centrifuge again at a higher speed until all of the solution has passed through.

5. If the sample volume was greater than 140 µl, repeat previous step until all of the lysate has been loaded onto the spin column.

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

   Note: It is not necessary to increase the volume of BWB1 even if the original sample volume was larger than 140 µl.

7. Add 500 µl BWB2, then centrifuge at full speed 20,000 x g (14,000 rpm) for 3 min. Continue directly with step 11, or to eliminate possible Buffer AW2 carryover, perform step 10 and then continue with step 11.

   Note: Residual BWB2 in the eluate may cause problems in downstream applications. Place the HiPure DR column in a new 2 ml collection tube (not provided), and discard the old collection tube with the flow-through. Centrifuge at full speed for 1 min.

8. Place the HiPure DR column in a clean 1.5 ml microcentrifuge tube (not provided). Discard the old collection tube containing the flow-through. Add 60 µl Buffer ERR equilibrated to room temperature into the HiPure DR column Close the cap, and incubate at room temperature for 1 min.
9. Centrifuge at 6000 x g (8000 rpm) for 1 min.

Note: A single elution with 60 µl Buffer ERR is sufficient to elute at least 90% of the viral RNA from the HiPure DR column. Performing a double elution using 2 x 40 µl Buffer ERR will increase yield by up to 10%. Elution with volumes of less than 30 µl will lead to reduced yields and will not increase the final concentration of RNA in the eluate.

Note: Viral RNA is stable for up to one year when stored at –30 to –15°C or at –90 to –65°C.