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## 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 $\mu$ l	200 $\mu$ l

## Protocol 1

### *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).
- If RNase treatment is desired, Prime-RNase can be ordered separately from ROJETechnologies, Cat NO. EB983013.
- Do not forget to add the appropriate amount of Ethanol (96–100%) to BW1 and BW2 buffers (BWB1 and BWB2) as indicated on the bottle, before using for the first time.
- If GL Buffer (GLB) forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

#### Process

1. Add 25  $\mu$ l RJ-Protease to a 1.5 ml clean microcentrifuge tube. Add 200  $\mu$ l sample (plasma, serum, body fluid and etc.) to the tube. Then add 200  $\mu$ l GLB. Pulse vortex for 15 s and incubate at 56 °C for 12 min.

2. Add 200  $\mu$ l Ethanol (96-100%) to the lysate, mix by pulse vortexing for 15 s, then centrifuge briefly.
3. Gently, pipette the mixture to a HiPure DR column placed in a 2 ml collection tube (supplied in the kit box). Centrifuge at 8000 rpm for 1 min. Discard flow-through and place back the HiPure DR column in to the collection tube.
4. Add 500  $\mu$ l BWB1 and centrifuge for 1 min at 8000 rpm, discard both the flow-through and the collection tube. Place back the HiPure DR column in to the collection tube.
5. Add 500  $\mu$ l BWB2 and centrifuge for 3 min at 14000 rpm. Discard both the flow-through and the collection tube. Place the HiPure DR column in a new clean 1.5 ml microcentrifuge tube (not provided).

**Note:** To avoid Ethanol carry over, be careful that the column does not come into contact with the flow-through, if it happens discard the flow-through, place the column back in a collection tube and centrifuge for another 1 min at 14000 rpm.

6. Pipette 30-50  $\mu$ l RRB directly onto HiPure DR column. Incubate at room temperature for 1-5 min. Centrifuge it at 12000 rpm for 1 min.