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
Blood*	Up to 250 µl	250 µl
Cultured cell	≤5 x 10^6 cells	250 µl
Buffy coat	Up to 200 µl	200 µl

*White Blood Cell counts are extremely variable from a person to person and therefore, for optimum results its recommended to determine cell counts for choosing the best protocol for purification. For healthy individuals, white blood cell counts normally from 4-10 million per 1 ml blood; however, depending on disease state, cell counts may be higher or lower than the normal range.

Protocol 1

Isolation of Genomic DNA (based on silica technology)

Sample type:

- Animal blood
- Cells
- 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 µl RJ-Protease to a 1.5 ml clean microcentrifuge tube. Add 250 µl blood (plasma, serum, body fluid and etc.) to the tube. Then add 250 µl GLB. Pulse vortex for 15 s and incubate at 56 °C for 12 min.

   Note: For cell pellets, add 250 µl GLB directly to the pellet or alternatively, for direct lysis of cells grown in a monolayer, add the appropriate amount of GLB to the cell-culture dish (refer to Table). Collect the lysate with a rubber policeman. Transfer the lysate into a microcentrifuge tube. Vortex to mix, and ensure that no cell clumps are visible.

   Optional: This protocol is specialized for DNA isolation. For usual and routine applications RNase treatment isn't needed. If RNA-free genomic DNA is required, add 10 µl Prime-RNase A (Cat NO. EB983013), mix by vortexing, and incubate for 5 min at room temperature before going to step 2.

2. Add 250 µl 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 the HiPure DR column in new collection tube (provided in the kit box).

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

4. Add 600 µl BWB1 and centrifuge for 1 min at 8000 rpm, discard the flow-through and place the HiPure DR column in the previous collection tube.
5. Add 600 µl 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 50-200 µl RRB directly onto HiPure DR column. Incubate at room temperature for 1-5 min. Centrifuge it at 8000 rpm for 1 min.

   Note: If the expected DNA yield is more than the yield from pervious step, put the HiPure DR column on a new microtube and add another 50-200 µl RRB, incubate for 5 min at room temperature. Then, centrifuge for 1 min at 8000 rpm. However, it is possible to pass the follow-through from step 6 once more to obtain DNA with higher concentrations.

Protocol 2

Isolation of Genomic DNA (based on silica technology)

Sample type: Buffy coat

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 µl RJ-Protease to a 1.5 ml clean microcentrifuge tube. Add 200 µl Buffy coat to the tube. Then add 200 µl GLB. Pulse vortex for 15 s and incubate at 56 °C for 12 min.

   Optional: This protocol is specialized for DNA isolation. For usual and routine applications RNase treatment isn't needed. If RNA-free genomic DNA is required, add 10 µl Prime-RNase A (Cat NO. EB983013), mix by vortexing, and incubate for 5 min at room temperature before going to step 2.

2. Add 200 µl 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 13000 rpm for 1 min. Discard flow-through and place the HiPure DR column in new collection tube (provided in the kit box).

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

4. Add 600 µl BWB1 and centrifuge for 1 min at 13000 rpm, discard the flow-through and place the HiPure DR column in the previous collection tube.
5. Add 600 µl 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 50-200 µl RRB directly onto HiPure DR column. Incubate at 56 ˚C for 1-5 min. Centrifuge it at 13000 rpm for 1 min.

   Note: If the expected DNA yield is more than the yield from pervious step, put the HiPure DR column on a new microtube and add another 50-200 µl RRB, incubate for 5 min at 56 ˚C. Then, centrifuge for 1 min at 13000 rpm. However, it is possible to pass the follow-through from step 6 once more to obtain DNA with higher concentrations.