Quick Protocol

DNJia Blood & Cell Kit

DNA isolation based on silica technology

- MiniPrep

For DNA Isolation from

Blood
Buffy coat
Cultured cells
Body fluid
Serum
Plasma
Buccal cells

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Kit Content

Component	50 preps	100 preps
GLB	12.5 ml	25 ml
BWB1 (concentrate)	16 ml	2 x 16 ml
BWB2 (concentrate)	16 ml	2 x 16 ml
RRB	10 ml	20 ml
RJ-Protease	1 ml	2 x 1 ml
HiPure OD Column	50	100
Collection Tube	100	200

Recommended Starting Material

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

Table 1: Recommended starting material and Lysis Buffer amount

Sample	Size of Starting Material	Lysis Buffer Amount
Blood*	Up to 250 µl	250 µl
Buffy coat	Up to 200 µl	200 µl
Cultured cell	≤5 x 10^6 cells	250 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.

Before Start

- If GLB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved. This is due to storage condition and will not influence the efficiency of buffer.
- Not forget to add the appropriate amount of ethanol (96–100%) to BWB1 and BWB2 as indicated on the bottle, before using for the first time, refer to washing buffer preparation.

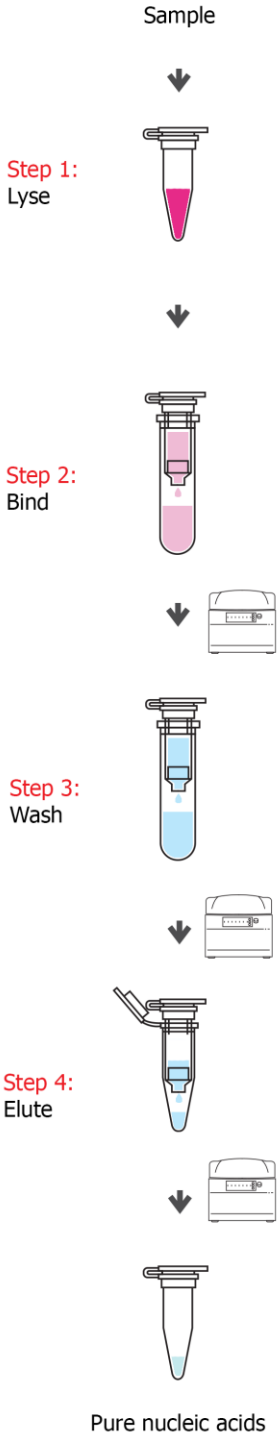
Washing Buffer Preparation

Before the first use, add appropriate amount of ethanol (96-100%) to each washing buffer tube, then mix thoroughly to prepare washing buffer, refer to Table 2. Do not forget to tick the check box on the bottle label to indicate that ethanol has been added. Before each use mix reconstituted buffer by shaking. Storing at room temperature.

Table 2: Washing buffer preparation

Buffer Name	Concentrated Volume	Amount of Ethanol	Final Volume
BWB1	16 ml	24 ml	40 ml
BWB2	16 ml	24 ml	40 ml

Procedure of silica-based DNA isolation in quick look



Protocols

Protocol 1: Isolation of Genomic DNA (Animal cell, Cells, Body fluid, Serum and plasma)

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 BWB1 and BWB2 as indicated on the bottle, before using for the first time, refer to washing buffer preparation part.
- If GLB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Process

1. Add 20 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. 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 OD 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 OD 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 OD 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 OD 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 OD 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 OD 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 (Buffy coat)

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 A can be ordered separately from ROJETechnologies, Cat No. EB983013.
- Do not forget to add the appropriate amount of ethanol (96–100%) to BWB1 and BWB2 as indicated on the bottle, before using for the first time, refer to washing buffer preparation part.
- If GLB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Process

1. Add 20 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 OD 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 OD 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 OD 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 OD 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 OD Column. Incubate at 56 ˚C for 3-5 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 OD 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.