Quick Protocol

DNJia Micro Kit

- MiniPrep

For DNA Isolation from

Amniotic Fluid

Semen

For Cell-free DNA Isolation from

Blood
Serum
Plasma
Cells
Body fluid
Semen

Kit Content

Component	100 preps
GLB	60 ml
BWB1 (concentrate)	2 X 16 ml
BWB2 (concentrate)	2 X 16.5 ml
RRB	20 ml
RJ-Protease	4 x 1.5 ml
Micro Column	100
Collection Tube	300
Carrier RNA	620 µg

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 including:

Sample	Size of Starting Material	Lysis Buffer Amount
Amniotic fluid	5-6 ml	350 µl
Blood, plasma, serum or body fluid	600 µl	600- 700 µl
sperm	300 µl	480 µl

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.

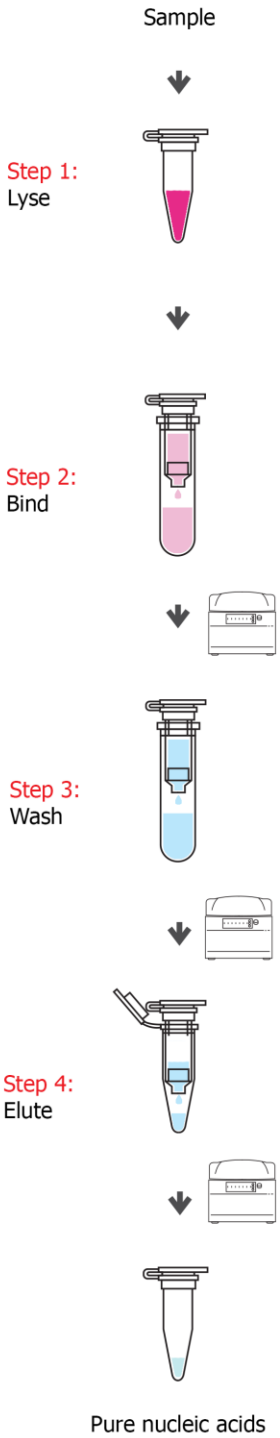
Washing Buffer Preparation

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	16.5 ml	38.5 ml	55 ml

Procedure of silica-based DNA isolation in quick look



Some tips to know:

- All centrifugation steps are carried out at room temperature (15–25°C).
- 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.
- If GLB forms precipitate, please warm it to 56°C until the precipitate has fully dissolved.

Protocol 1

- ***Isolation of Genomic DNA from Amniotic fluid (based on silica technology)***

Process

1. Centrifuge 1.5 ml of the amniotic fluid for 10 min at 5000 rpm in a clean microcentrifuge tube, discard the supernatant and repeat this step until you pellet 5-6 ml amniotic fluid.
2. Add 350 µl GLB to the pellet then 35 µl RJ-Protease. Pulse vortex until the pellet is dissolved thoroughly incubate at 56 °C for 20 min, pulse vortex for 15 s every 6 min during the incubation.
3. Add 350 µl ethanol (96-100%) to the lysate, mix by pulse vortexing for 15 s, then centrifuge briefly.
4. Gently, pipette the mixture to a Micro 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 Micro Column in to the collection tube.
5. Add 650 µl BWB1 and centrifuge for 1 min at 8000 rpm, discard both the flow-through and the collection tube. Place back the Micro Column in to the collection tube.
6. Add 750 µl BWB2 and centrifuge for 15 s at 10000 rpm. discard both the flow-through and the collection tube. Place back the Micro Column in to the collection tube.
7. Add 500 µl BWB2 and centrifuge for 3 min at 14000 rpm. Discard both the flow-through and the collection tube. Place the Micro 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.

8. Pipette 25-50 µl RRB directly onto Micro Column. Incubate at room temperature for 5 min. Centrifuge it at 12000 rpm for 2 min.

Protocol 2

- ***Isolation of genomic and Cell-Free DNA from semen***

A) Isolation of Genomic DNA from sperm

Sample Type: Semen

Some tips to know:

- All steps, before applying the sample to Micro Column, are carried out on the ice. Before using the protocol for the first time, add 2-Mercaptoethanol to GLB, 4% of GLB volume. It is better to calculate it every time you test and add 4% of 2ME to it for higher efficiency. It is 480 µl of GLB and 20 µl of 2ME for each sample test. 2-Mercaptoethanol is commercially available and due to safety issues not provided in the kit.
- Before using the protocol for the first time, add isopropanol to TLB, 40% of TLB volume. It is better to calculate it every time you test and add 40% of isopropanol to it for higher efficiency. It is 300 µl of TLB and 200 µl of isopropanol for each sample test. Isopropanol is commercially available and due to safety issues not provided in the kit.

Attention: It is recommended to prepare it freshly. It is not stable for more than 2 weeks.

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

Process

1. Add 75 µl RJ-Protease to a 1.5 ml clean microcentrifuge tube. Add 300 µl semen to the tube. Then add 500 µl GLB (prepared 2-ME). Pulse vortex for 1 min and incubate at 60 °C for 2h. Every 10 minutes invert it 20 times Pulse vortex for 3s.
2. Add 500 µl TLB (prepared isopropanol). Pulse vortex for 20s and incubate at 4 °C for 3min then incubate at 70°C for 1min. Transfer up to 700 µl of the sample, including any precipitate that may have formed, to a Micro Column placed in a 2ml collection tube (supplied in the kit box). Close the lid gently, and centrifuge for 1min at 8000 rpm. Discard the flow-through. Reuse the collection tube in step 10

3. Add the remainder of the sample to the column and centrifuge for 1min at 8000 rpm Discard the flow-through new collection.
4. Add 650 µl BWB1 to the Micro Column. Centrifuge for 1min at 8000 rpm at room temperature. Discard the flow-through.
5. Add 750 µl BWB2 to the Micro Column. Centrifuge for 3min at 14000 rpm at room temperature. Discard the flow-through new collection Centrifuge for 1 min at 14000 rpm at room temperature. Discard the collection tube with the flow-through.
6. Place the Micro Column in a new 1.5 ml microtube. Add 50µl RRB directly to the Micro Column membrane and incubation in RT for 3-5min then Centrifuge for 75s at 13000 rpm.

B) Isolation of Cell Free DNA from semen

Sample Type: Semen

Some tips to know:

- All steps, before applying the sample to Micro Column, are carried out on the ice. Before using the protocol for the first time, add 2-Mercaptoethanol to GLB, 4% of GLB volume. It is better to calculate it every time you test and add 4% of 2ME to it for higher efficiency. It is 480 µl of GLB and 20 µl of 2ME for each sample test. 2-Mercaptoethanol is commercially available and due to safety issues not provided in the kit.
- Before using the protocol for the first time, add isopropanol to TLB, 40% of TLB volume. It is better to calculate it every time you test and add 40% of isopropanol to it for higher efficiency. It is 300 µl of TLB and 200 µl of isopropanol

for each sample test. Isopropanol is commercially available and due to safety issues not provided in the kit.

Attention: It is recommended to prepare it freshly. It is not stable for more than 2 weeks.

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

Process

1. Add 500 µl of sperm sample into a vial and put in a centrifuge at 900 × g for 10 minutes. Then, transfer the supernatant to the sterile 1.5 vials.
2. Add 90 µl RJ-Protease and 15 µl RNA carriers. Then add 500 µl GLB (prepared 2-ME). Pulse vortex for 30s and incubate at 60 °C for 1h. Every 10 minutes invert it 20 times Pulse vortex for 3s.
3. Add 400 µl TLB (prepared isopropanol). Pulse vortex for 20s and incubate at 4°C for 5 min then incubate at 70°C for 1min. Transfer up to 700 µl of the sample, including any precipitate that may have formed, to a Micro Column placed in a 2ml collection tube (supplied in the kit box). Close the lid gently, and centrifuge for 30min at 12000 rpm. Discard the flow-through. Reuse the collection tube.
4. Add the remainder of the sample to the column and centrifuge for 30s at 12000 rpm Discard the flow-through new collection.
5. Add 650 µl BWB1 to the Micro Column. Centrifuge for 30s at 12000 rpm at room temperature. Discard the flow-through.
6. Add 750 µl BWB2 to the Micro Column. Centrifuge for 3min at 14000 rpm at room temperature. Discard the flow-through new collection Centrifuge for 1 min at 14000 rpm at room temperature. Discard the collection tube with the flow-through.
7. Place the Micro Column in a new 1.5 ml microtube. Add 30µl RRB directly to the Micro Column membrane and incubation in RT for 5min then Centrifuge for 2min at 14000 rpm.

Protocol 3

Isolation of Cell free DNA (based on silica technology)

Sample type:

- Animal blood
- Cells
- Body fluid
- Serum
- Plasma

Process

1. Add 60 µl RJ-Protease to a 2 ml clean microcentrifuge tube. Add 600 µl blood (plasma, serum or body fluid and etc.) to the tube. Then add 600 µl GLB. Add 6 µl RNA carrier to the tube. Pulse vortex for 20 s and incubate at 60 °C for 20 min.

   Note: For cell pellets, add 700 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 CF 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 7 min at room temperature before going to step 2.
2. Add 600 µl ethanol (100%) to the lysate, mix by pulse vortexing for 20 s, then centrifuge briefly.
3. Gently, pipette half of the mixture to a Micro Column placed in a 2 ml collection tube. Centrifuge at 8000 rpm for 1 min. Discard flow-through and place the Micro Column in the collection tube.

   Note: If the lysate did not pass the column, centrifuge at full speed until it passes through the column.
4. Gently, pipette the remaining mixture to the Micro Column placed in the 2 ml collection tube. Centrifuge at 8000 rpm for 1 min. Discard flow-through and place the Micro Column in the collection tube.

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

passes through the column.

5. Add 650 µl BWB1 and centrifuge for 1 min at 8000 rpm, discard the flow-through and place the Micro Column in the previous collection tube.
6. Add 750 µl BWB2 and centrifuge for 3 min at 14000 rpm. Discard both the flow-through and the collection tube. Place the Micro 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 flowthrough, place the column back in a collection tube and centrifuge for another 1 min at 14000 rpm.
7. Pipette 30 µl RRB directly onto Micro Column. Incubate at room temperature for 3-5 min. Centrifuge it at 12000 rpm for 1 min.