WelPrep
DNA Isolation Kit

For DNA purification from blood, tissue and cultured cell.

For Research Use Only,
Not For Use In Diagnostic Procedures.

Our Website
www.welgene.com.tw

Content

Kit contents

Items	Quality
WelPrep DNA stabilizer	38 ml
DNA Lysis buffer	8.4 ml
DNA Binding buffer	8.4 ml
DNA Wash buffer 1	22 ml
DNA Wash buffer 2*	4.4 ml
Poteinase K Solution	0.9 ml
Nuclease-free H2O	3.4 ml
DNA Spin column	40
Hand book	1

2*. Before use, please add 17.6 ml of 100% EtOH first.

Storage

The buffers and DNA Spin Columns can be stored dry at room temperature (15–25°C) for up to 2 years.
Proteinase K solution is stable for at least one year after delivery when stored at 4°C.

Product Use Limitations

For research use only. Not for use in diagnostic procedures. The performance characteristics of this product have not been fully established.

Principle

The WelPrep DNA Kit allows the isolation of total DNA from various biological samples. The procedure is based on the selective binding properties of a silica-based membrane. DNA lysis buffer, which contain ionic detergent disrupts cells, denatures cellular proteins. After lysis with buffer, samples are mixed with DNA binding buffer, and ethanol subsequently to optimize the binding conditions. The resulting solution is applied to a WelPrep DNA spin column. During a brief centrifugation, DNA is adsorbed by the silica membrane of the spin column and other impurities pass through. Remaining contaminants are removed in three wash steps, and pure DNA is then eluted by nuclease-free water.

Equipment and Reagents to Be Supplied by Users

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective gloves.

- RNase (20 mg/ml, optional)
- Ethanol (100%)
- 1.5 ml or 2 ml microcentrifuge tubes
- Sterile, DNase-free pipet tips
- Variable-speed microcentrifuge with a rotor for 2 ml microcentrifuge tubes
- Heating block, or water bath
- Vortex mixer
- Disposable gloves

Protocol

I. Before start :

The protocol is optimized for isolating DNA from the following sample amount:

Sample type	Sample amount
blood	300 µl
tissue	25 mg
cell	~1x10^6

II. Protocol :

1. Disrupt cells

A. Blood

A-1. Transfer 300 µl of blood to a 1.5-ml tube.

A-2. Add 900 µl of WelPrep DNA Stabilizer, and mix thoroughly by hand shaking.

A-3. Incubate the sample at room temperature for 5 min, and centrifuge at 8000 rpm for 10 min.

A-4. Remove the supernatant completely.

A-5. Add 200 µl of DNA Lysis buffer and 20 µl of Proteinase K solution. Mix the solution thoroughly by hand shaking.

A-6. Incubate the sample at 65°C with occasionally mixing until the solution becomes clear.

Note : Lysis time varies depending on the cell number. It usually takes about 1 hour to get the sample lyzed completely.

B. Tissues

B-1. Excise up to 25 mg of tissue, and cut it into small pieces, place in a 1.5-ml tube.

B-2. Add 200 µl of DNA Lysis buffer and 20 µl of Proteinase K solution. Mix the solution thoroughly by hand shaking.

Note : Cut it as small as possible, the diameter of each piece should be less than 0.3 cm at least.

B-3. Incubate the sample at 65°C with occasionally mixing until the solution becomes clear.

Note: Lysis time varies depending on the tissue type. It usually takes about 1 hour to get the sample lyzed completely.

C. Cells

C-1. Pellet cells by centrifugation for 5 min at 500 x g. Discard the supernatant.

C-2. Add 200 µl of DNA Lysis buffer and 20 µl of Proteinase K solution. Mix the solution thoroughly by hand shaking.

C-3. Incubate the sample at 65°C with occasionally mixing until the solution becomes clear.

Note: Lysis time varies depending on the cell number. It usually takes about 1 hour to get the sample lyzed completely.

2. [Optional] after the solution is cool down to room temperature, add 4 µl of 20 mg/ml RNase A solution and incubate samples at 37°C for 1 hour.

3. Add 200 µl of DNA binding buffer, and mix thoroughly by hand shaking.

Note: It is normal that the solution becomes cloudy after the DNA binding buffer is added to the sample.

4. Add 200 µl of 100% ethanol, and mix thoroughly by hand shaking.

5. Transfer sample mixture onto DNA spin column, centrifuge at 13000 rpm for 60 sec. (Discard the flow-through from the collection tube).

6. Pipette 500 µl DNA wash buffer 1 onto the column, centrifuge at 13000 rpm for 60 sec. (Discard the flow-through from the collection tube).

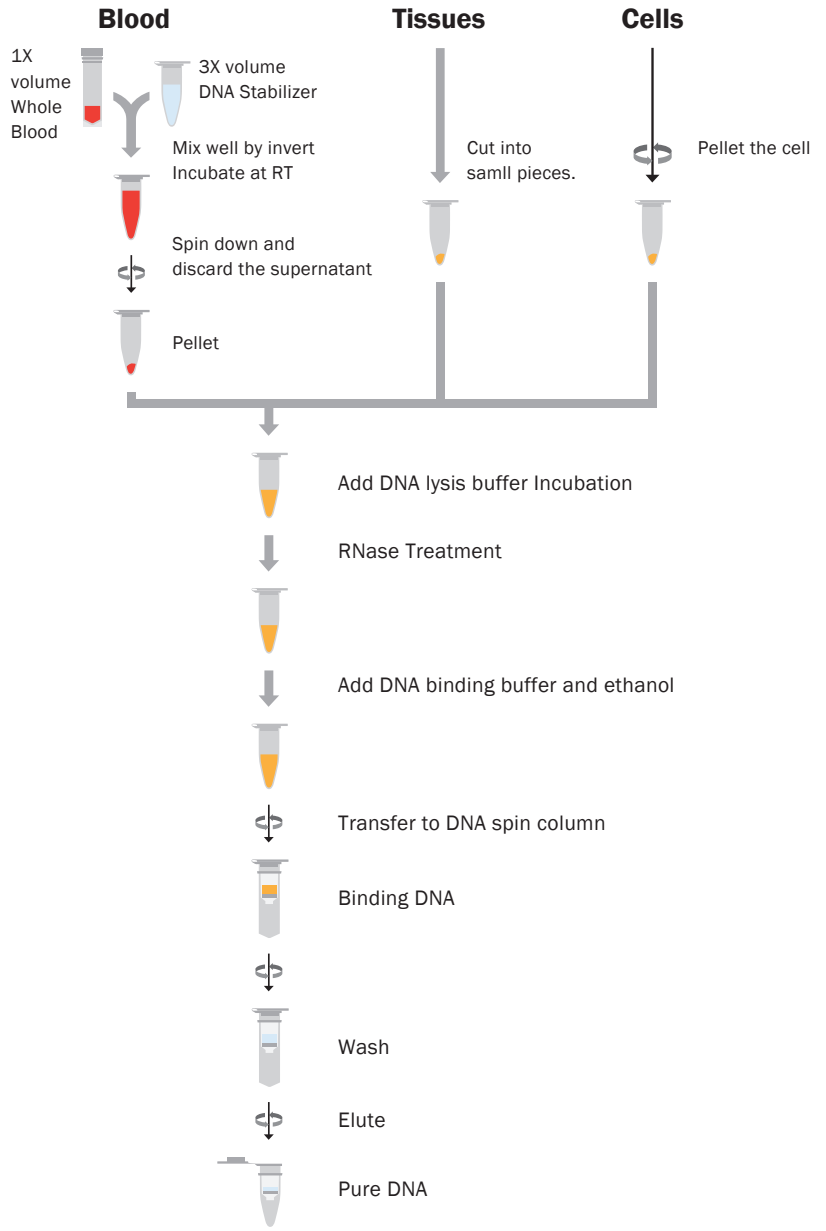
7. Pipette 500 µl of DNA wash buffer 2 onto the column, centrifuge at 13000 rpm for 30 sec. (Discard the flow-through from the collection tube).

8. Transfer the column in a new 2 ml collection tube, and discard the old collection tube containing the flow-through. Centrifuge at full speed for 1 min.

9. To elute the DNA, transfer the column on a new 1.5 ml elution tube, and pipette 40 µl nuclease-free water onto the column membrane and incubate at 65°C for 5 min, then centrifuge at 13000 rpm for 1 min.

10. Repeat the step 9 again.

WelPrep DNA procedure



Troubleshooting Guide

Comments and suggestions	
Low DNA yields	
a) Inefficient cell lysis due to insufficient mixing with DNA Lysis Buffer	Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and DNA Lysis Buffer immediately and thoroughly by vortexing.
b) No ethanol or low percentage of ethanol added to the lysate	Prepare a new ethanol and do not use denatured alcohol, which contains other substances such as methanol or methyl ethyl ketone. Repeat the purification procedure with a new sample.
c) WelPrep DNA spin column not incubated at 65°C for 5 min	After addition of water, the DNA spin column should be incubated at 65°C for 5 min.
d) DNA not eluted efficiently	To increase elution efficiency, add water onto the center of the DNA spin column and incubate the column for5 min at 65°C before centrifugation.
e) Wash Buffer 2 prepared incorrectly	Check that Buffer Wash Buffer 2 concentrates were diluted with the correct volumes of pure ethanol. Repeat the purification procedure with a new sample.
f) Wash Buffer 1 and 2 used in the wrong order	Make sure that Wash Buffer 1 and 2 are used.
Low OD260/280 raio	
a) Inefficient cell lysis due to insufficient mixing with DNA Lysis Buffer	Repeat the DNA purification procedure with a new sample. Be sure to mix the sample and DNA Lysis Buffer immediately and thoroughly by vortexing.

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Troubleshooting Guide

Comments and suggestions	
b) No ethanol or low percentage of ethanol added to the lysate	Prepare a new ethanol and do not use denatured alcohol, which contains other substances such as methanol or methyl ethyl ketone. Repeat the purification procedure with a new sample.
c) Wash Buffer 2 prepared incorrectly	Check that Buffer Wash Buffer 2 concentrates were diluted with the correct volumes of pure ethanol. Repeat the purification procedure with a new sample.
d) Wash Buffer 1 and 2 used in the wrong order	Make sure that Wash Buffer 1 and 2 are used.
Others	
Clogged membrane	Cell number > 5 x 106/200 µl usually clogs the membrane of spin column. Dilute the sample with PBS and repeat the purification.

Note

Note

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