WelPrep

Plasma DNA Isolation Kit

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

Our Website
www.welgene.com.tw

Content

Kit contents

Items	Quality
Digestion Buffer	25 ml
Pwash Buffer 1	25 ml
Pwash Buffer 2*	5 ml
Poteinase K Solution	3.8 ml
Nuclease-free H_2O	3.4 ml
WelPrep DNA Mini Spin column	40
Collection Tubes (2ml)	40
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2*. Before use, please add 20 ml of 100% EtOH first.

Storage

The buffers and DNA Mini Spin Columns can be stored dry at room temperature (15–25°C) for up to 2 years.
Proteinase K solution should be stored dry at -20°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%)
- 15 ml conical centrifuge tubes
- 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
- Carrier RNA (optional, usage of carrier RNA can increase the DNA yield) (Qiagen#1068337, 1 vial per kit; Qiagen#1017647, 12 vials per kit)

Protocol

I. Before start :

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

Sample type	Sample amount
plasma	1 ml

2. Preparation of carrier RNA (optional):
   - Place the carrier RNA dry pellet (310 µg) in the centrifuge for 5 min at 12000 rpm.
   - Open the lid carefully, add 310 µl Digestion buffer, then briefly centrifuge. When pellet is dissolved and solution can be stored in -20°C. (The storage solution should avoid repeat thawing and refreezing of samples no more than 3 times.).

II. Protocol :

1. Prepare a 15 ml conical centrifuge tube. Pipet 90 µl Proteinase K Solution into the tube bottom.
2. Add 900µl plasma sample to the tubes. Mix by vortexing and spin down.
3. Add 900µl Digestion Buffer and 9 µL Carrier RNA (usage of carrier RNA is optional) to the tubes. Mix by vortexing and spin down.
4. Incubate at 60°C for 60 min.
5. Add 900 µl ethanol (100%) to each tube mixture. Mix by hand shaking and spin down.
6. Carefully Transfer proper mixture to the WelPrep DNA Mini Spin Column (in a 2 ml collection tube). Close the cap and centrifuge at 8,000 rpm for 2 min. (Discard the flow-through from the collection tube). Repeat this step until all mixture pass through the spin column.
7. Add 600 µl Pwash Buffer 1 to the WelPrep DNA Mini Spin Columnn and centrifuge at 13,000 rpm for 1 min. (Discard the flow-through from the collection tube)
8. Add 600 µl Pwash Buffer 2 to the WelPrep DNA Mini Spin Column and centrifuge at 13,000 rpm for 1 min. (Discard the flow-through from the

collection tube)

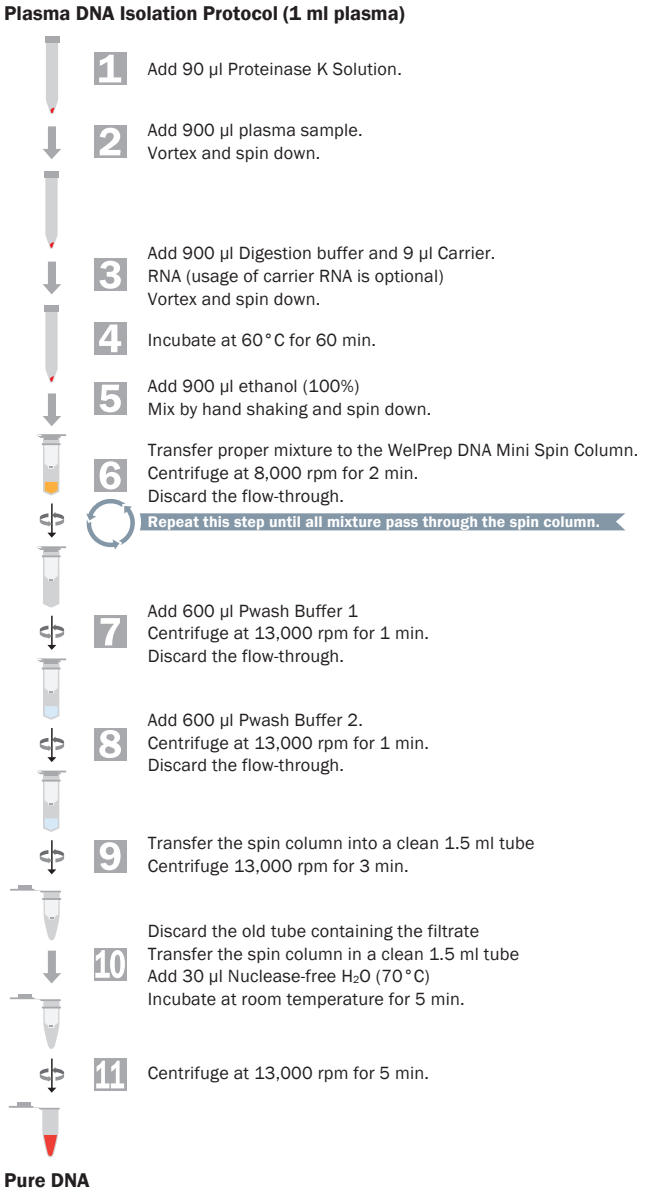
9. Transfer the WelPrep DNA Mini Spin Column into clean 1.5 ml tube and centrifuge 13,000 rpm for 3 min.
10. To elute the DNA, Transfer the WelPrep DNA Mini Spin Column in a clean 1.5 ml tube and discard the old tube containing the filtrate. Add 30 µl Nuclease-free H_2O (70°C) to the WelPrep DNA Mini Spin Column and incubate at room temperature for 5 min.
11. Centrifuge at 13,000 rpm for 5 min.

Appendix 1.

Plasma Sample Preparation:

1. Fresh blood was collected into BD Vacutainer EDTA tubes
2. Instantly mix by hand shake.
3. Transfer the blood sample to a clean 15 ml centrifuge tube (#1).
4. Centrifuge at 4°C for 12 minutes at 1500 × g, and carefully transfer the plasma layer to a new clean 15 ml centrifuge tube (#2).
   This will give three layers: (from top to bottom) plasma, leucocytes (buffy coat), erythrocytes.
5. [Optional] Centrifuge this 15 ml centrifuge tube (#2) at 4°C for 12 minutes at 1500 × g, and carefully transfer the supernatant to a new clean 15 ml centrifuge tube (#3).
6. Processed plasma samples were stored at –80°C until DNA was extracted.

WelPrep Plasma DNA procedure



Troubleshooting Guide

Comments and suggestions	
Low DNA yields	
a) Inefficient protein digestion due to insufficient mixing with Digestion Buffer	Repeat the DNA purification procedure with a new sample. Be sure to add proteinase K first, then add the plasma and Digestion Buffer. Mix the sample and Digestion 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) DNA not eluted efficiently	To increase elution efficiency, add water onto the center of the DNA Spin Column and incubate the column for 5 min at 70°C before centrifugation.
d) Pwash Buffer 2 prepared incorrectly	Check that Pwash Buffer 2 concentrates were diluted with the correct volumes of pure ethanol. Repeat the purification procedure with a new sample.
e) Pwash Buffer 1 and 2 used in the wrong order	Make sure that Pwash Buffer 1 and 2 are used.

Troubleshooting Guide

Comments and suggestions	
Low OD260/280 raio	
a) 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.
b) Pwash Buffer 2 prepared incorrectly	Check that Pwash Buffer 2 concentrates were diluted with the correct volumes of pure ethanol. Repeat the purification procedure with a new sample.
c) Pwash Buffer 1 and 2 used in the wrong order	Make sure that Pwash Buffer 1 and 2 are used.

Order Information

Kit		
D010	WelPrep DNA Isolation Kit	40 Rxns
D015	WelPrep Plasma DNA Isolation Kit	40 Rxns
R001	WelPrep Blood RNA Kit	40 Rxns
R002	WelPrep Cell/ Tissue RNA Kit	40 Rxns

Component		
W007	WelPrep DNA Stabilizer	38 ml
W019	DNA Lysis Buffer	8.4 ml
W018	DNA Binding Buffer	8.4 ml
W010	DNA Wash Buffer 1	22 ml
R003	Shredder Column	40 Rxns
R004	Blood RNA Stabilizer	260 ml
W002	RNA Lysis Buffer	10 ml
W003	RNA Wash Buffer 1	22 ml

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Note

Our Website www.welgene.com.tw