# *WelPrep* Blood RNA Kit

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# Content

## Kit contents

| Items | Quantity |
|---|---|
| *WelPrep* Blood RNA stabilizer | 260 ml |
| RNA Lysis buffer | 10 ml |
| Proteinase K | 1 ml |
| Shredder column | 40 |
| RNA spin column | 40 |
| RNA Wash buffer 1* | 22 ml |
| RNA Wash buffer 2* | 9 ml |
| Nuclease-free $H_2O$ | 1 ml |
| Hand book | 1 |

1*. Before use, please add 9.5 ml of 100% EtOH first. The mild turbid wash buffer 1 normally becomes clear after add alcohol.

2*. Before use, please add 36 ml of 100% EtOH first.

## Storage

The buffers and RNA Spin Columns can be stored dry at room temperature (15–25°C) for up to 2 year. Proteinase K solution that 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* Blood RNA Kit allows the isolation of total RNA from 2.5 ml human whole blood samples. The procedure is simple (see Figure 1 , page 4). The beginning centrifugation step pellets nucleic acids. The pellet is washed, resuspended, and incubated in Proteinase K solution to digest proteins. A second centrifugation step is carried out to remove residual cell debris, and the supernatant is transferred to a shredder column to reduce the viscosity of the solution. Following on-column DNase digestion is to remove residual DNA and is recommended for RNA applications that are sensitive to DNA contamination. Ethanol is added to optimize the binding conditions, and the solution is applied to a *WelPrep* RNA spin column. During a brief centrifugation, RNA 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 RNA is then eluted by nuclease-free water.

Using the *WelPrep* Blood RNA System, typical yields of RNA isolated from 2.5 ml human whole blood are between 4 and 12 µg. However, the yield is highly donor-dependent, and in some cases, higher or lower yields may be achieved (Figure 1).

Purity and quality of these RNA are evaluated by OD 260/280 ratio, 28S/18S rRNA ratio and RIN (RNA Integrity Number), (Figure 1). The data show overall OD 260/280 > 2.0, average 28S/18S rRNA ratio = 1.4, RIN = 7.8.

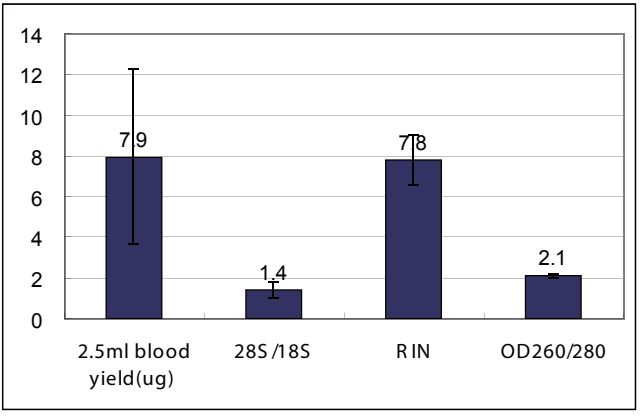



Figure 1
Yields of RNA isolated from 2.5 ml human whole blood are between 4 and 12 µg. Purity and quality of these RNA are evaluated by OD 260/280 ratio, 28S/18S rRNA ratio, RIN (RNA Integrity Number).

## Equipment and Reagents to Be Supplied by Users

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

- blood tubes with EDTA as anti-coagulant.
- autoclaved 2d. $H_2O$
- chloroform
- Ethanol (96–100%)
- DNase I
- 1.5 ml or 2 ml microcentrifuge tubes
- Sterile, RNase-free pipet tips
- Variable-speed microcentrifuge with a rotor for 2 ml microcentrifuge tubes
- Centrifuge capable of attaining 3000–5000 x g and equipped with a fixed angel rotor for 50-ml centrifugal tubes
- heating block, or water bath
- Vortex mixer
- Disposable gloves

# Protocol: Isolation of RNA from Whole Blood Collected into *WelPrep* Blood RNA Tubes

## I. Before start:

- The protocol is for isolating total RNA from 2.5ml whole blood samples.
- The blood RNA stabilizer shall be stored in a dry environment at room temperature.
- After blood collection, blood-reagent mixture can be stored at 4°C at least for one week. For longer term storage, tubes can be stored at -20°C or -70°C for up 6 months.

## II. Protocol

### A. Collection of Blood Samples:

1. Pipette 6.25ml of Blood RNA stabilization reagent into the fresh 50ml centrifuge tube.
2. Collect blood by method of choice into a syringe and immediately add 2.5ml into the centrifuge tube.
3. It is critical to then immediately invert the tube at least 10 times to assure complete mix with the reagents.
4. Further mix the tube with the rotator shaking at 11rpm at room temperature for 2 hours. The step is to lyze blood cells completely.
5. Transfer to -20°C or -80°C for longer storage if not immediately isolating RNA.

### B. RNA Isolation Protocol:

Things to do before starting

- Once the isolation protocol begun, it is important to continue through all the steps without any interruptions or hesitations.
- All centrifugation steps should be carried out at room temperature (15-25°C).
- If the tube is frozen, allowed the frozen solution to be thawed completely at a 22°C bath before the RNA isolation process.

1. Centrifuge the pretreated blood sample tube for 15min at 5000g.

   ***Note:*** *Be sure that the blood sample has been incubated for 2 hrs at room temperature to achieve complete lysis of the blood cells.*

2. Remove the supernatant by decanting. Add 6ml of autoclaved 2d.$H_2O$ water to the pellet. Vortex until the pellet is visibly dissolved, centrifuge for 15 min at 5000g.

3. Remove and discard the entire supernatant by decanting. Add 300ul of autoclaved 2d.$H_2O$ to the pellet, and thoroughly resuspend the pellet by pipetting.

4. Pipet the sample into a 1.5ml microcentrifuge tube, and add 210ul lysis buffer. Thoroughly mix the solution by vortexing, then add 24ul of Proteinase K.

5. Thoroughly mix the solution by vortexing, and incubate the tube at 55°C for 10 min. During the incubation period, vortex the tube once again.

6. Pipet the Proteinase K pretreated lysate directly onto shredder spin column, centrifuge at 13k rpm for 2 min. Carefully transfer the entire supernatant (without pellet) of the flow-through fraction onto the same shredder column, centrifuge at 13k rpm for 2min.

7. Carefully remove the flow-through into a new 1.5ml microcentrifuge tube without disturbing the pellet in the processing tube.

8. Add 210ul Chloroform and thoroughly mix well by shake, centrifuge at 13k rpm for 10 min.

9. Carefully remove the supernatant into a new 1.5ml microcentrifuge tube, which contains DNA, RNA, salts and protein debris. While remove as much as possible the supernatant into the tube, also avoid the debris at the water/organic interfaces.

10. Add 270ul 100% ethanol into the lysate then vortex and spin down.

11. Remove 700ul lysate onto RNA spin column, centrifuge at 13k rpm for 30 sec. (Discard the flow-through from the collection tube).

12. Pipette 350ul RNA wash buffer 1 onto the column, centrifuge at 13k rpm for 60 sec. (Discard the flow-through from the collection tube).

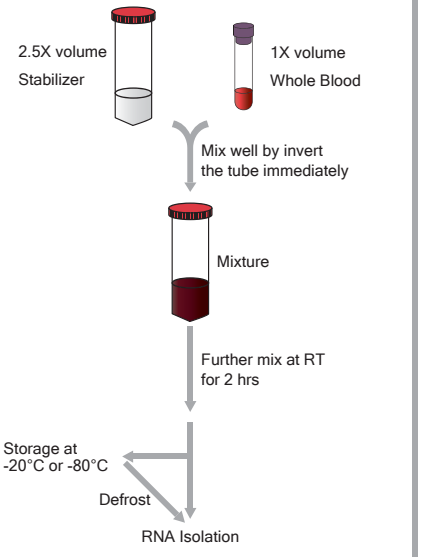
13. Pipette the 30 Units DNase I solution mix directly onto the spin-column membrane, and incubate at room temperature for 15 min.

14. Pipette 350ul of wash buffer 1 onto the column, centrifuge at 13k rpm for 30 sec. (Discard the flow-through from the collection tube).

15. Pipette 500ul of wash buffer 2 onto the column, centrifuge at 13k rpm for 30 sec. (Discard the flow-through from the collection tube).
16. Pipette 500ul of wash buffer 2 onto the column, centrifuge at 13k rpm for 2 min. (Discard the flow-through from the collection tube).
17. Place the column on a new 1.5ml elution tube, and pipette 30ul nuclease-free water onto the column membrane and incubate for 1min, then centrifuge at 13k rpm for 2 min.
18. Store the RNA samples at -80°C.

# *WelPrep* Blood RNA procedure

## A. Collection of Blood Sample




## B. RNA Isolation protocol

## Troubleshooting Guide

| | Comments and suggestions |
|---|---|
| RNA degraded | Be careful any RNases contamination in the container or tubes during the whole isolation procedure. Cleaning equipments by RNAZap (Ambion) inactivates contaiminated RNase. |
| RNA does not perform well in downstream experiments | |
| a) Salt carryover during elution | Be sure that Wash Buffer 1 is at room temperature. |
| b) Ethanol carryover during wash | Be sure that during the second wash with Wash Buffer 2, the *WelPrep* spin column is spun at maximum speed for 3 min to dry the spin column membrane. After centrifugation, remove the *WelPrep* spin column from the collection tube carefully so that the flow-through does not come into contact with the spin column, causing carryover of ethanol. Continue with the optional centrifugation in step 12 of the procedure. |
| Low RNA yield | |
| a) Less than 2.5 ml blood collected | Ensure that 2.5 ml blood is collected. |
| b) blood pellet not well-resuspended | Be sure that the suspension of blood pellet in the autoclaved 2d. $H_2O$ is complete in the step 2 of the protocol. |
| c) Incubation with Proteinase K is not at the correct temperature | Ensure that the shaker–incubator used in step 5 carried out at the correct set to 55°C. If using a heating block or water bath, temperature vortex the sample quickly during the incubation to minimize any cooling. |
| d) After blood mixed with *WelPrep* Blood RNA Stabilizer, blood is incubated and mixed for at least 2 hours | Incubation blood with the *WelPrep* Blood RNA Stabilizer should be for at least 2 hours after mixing. |

# Appendix: Storage, Quantification, and Determination of Quality of Total RNA

## Storage of RNA

Purified RNA may be stored at –20°C or –80°C in Nuclease-free water.

## Quantification of RNA

The concentration of RNA should be determined by measuring the absorbance at 260 nm (A260) in a spectrophotometer. To ensure significance, readings should be in the linear range of the spectrophotometer. An absorbance of 1 unit at 260 nm corresponds to 44 µg of RNA per ml (A260 = 1→44 µg/ml). This relation is valid only for measurements in 10 mM Tris•Cl, pH 7.5. Therefore, if it is necessary to dilute the RNA sample, this should be done in 10 mM Tris•Cl. As discussed below (see "Purity of RNA"), the ratio between the absorbance values at 260 and 280 nm gives an estimate of RNA purity. When measuring RNA samples, be certain that cuvettes are RNase-free, especially if the RNA is to be recovered after spectrophotometry. This can be achieved by washing cuvettes with 0.1 M NaOH,* 1 mM EDTA* followed by washing with RNase-free water . Use the buffer in which the RNA is diluted to zero the spectrophotometer.

An example of the calculation involved in RNA quantification is shown below:

Volume of RNA sample = 80 µl

Dilution = 10 µl of RNA sample + 140 µl 10 mM Tris•Cl, pH 7.5 (1/15 dilution).

Measure absorbance of diluted sample in a cuvette (RNase-free).

A260 = 0.3

Concentration of RNA sample = 44 x A260 x dilution factor

= 44 x 0.3 x 15

= 198 µg/ml

Total yield = concentration x volume of sample in milliliters

= 198 µg/ml x 0.08 ml

= 15.8 µg RNA

** When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier. WelPrep* Blood RNA Kit Handbook

### Purity of RNA

The ratio of the readings at 260 nm and 280 nm (A260/A280) provides an estimate of the purity of RNA with respect to contaminants that absorb in the UV, such as protein. However, the A260/A280 ratio is influenced considerably by pH. Lower pH results in a lower A260/A280 ratio and reduced sensitivity to protein contamination.* For accurate values, we recommend measuring absorbance in 10 mM Tris•Cl, pH 7.5. Pure RNA has an A260/A280 ratio of 1.9–2.1† in 10 mM Tris•Cl, pH 7.5. Always calibrate the spectrophotometer with the same solution.

### DNA contamination

No currently available purification method can guarantee that RNA is completely free of DNA, even when it is not visible on an agarose gel. To prevent any interference by DNA in RT-PCR applications, we recommend designing primers that anneal at intron splice junctions so that genomic DNA will not be amplified. Alternatively, DNA contamination can be detected on agarose gels following RT-PCR by performing control experiments in which no reverse transcriptase is added prior to the PCR step or by using intron-spanning primers. For sensitive applications, such as differential display, or if it is not practical to use splice-junction primers, DNase digestion of the purified RNA with RNase-free DNase is recommended.

The DNase is efficiently washed away in the subsequent wash steps.

Alternatively, after the *WelPrep* procedure, the elute containing the RNA can be treated with DNase.

Following heat inactivation of the DNase, the RNA can be used directly in downstream applications.

**Integrity of RNA**

The integrity and size distribution of total RNA purified using the *WelPrep* Blood RNA System can be checked by denaturing agarose gel electrophoresis and ethidium bromide staining . The respective ribosomal bands should appear sharp on the stained gel. 28S ribosomal RNA bands should be present with an intensity approximately twice that of the 18S RNA band. If the ribosomal bands in a given lane are not sharp, but appear as a smear of smaller sized RNAs, it is likely that the RNA sample suffered major degradation during preparation.

Size of Human Ribosomal RNAs

Human rRNA Size (kb)

18S 1.9

28S 5.0

* Wilfinger, W.W., Mackey, M., and Chomczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. BioTechniques 22, 474.

† Values up to 2.3 are routinely obtained for pure RNA (in 10mM Tris•Cl, pH 7.5) with some spectrophotometers.