(DP433) RNAprep Pure Blood Kit

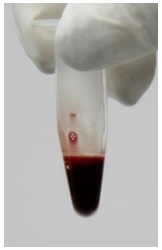
——Blood

TIANGEN BIOTECH(BEIJING)CO., LTD

Ver. No. 20170331

Experiment Preparation

1. Fresh blood sample (200 μl)
2. Ethanol, β-mercaptoethanol
3. Disposable sterile syringe (for DNase I preparation); Pipette and matched sterile RNase-Free tips (200 μl ,1 ml); 1.5 ml and 2.0 ml centrifuge tubes (RNase-free)
4. Fume hood; Vortex oscillator; Dry bath; Refrigerated centrifuge



Experiment Preparation - Kit Preparation 1

Please add 96-100% ethanol in Buffer RW before use according to the volume indicated on the label of the bottle, and label the tube.

Preparation of DNase I storage solution

Dissolve the DNase I powder (1500 U) in 550 μl RNase-Free ddH_2O, gently mix well, and store in -20℃ (for up to 9 months) after aliquoting.

Note: The thawed the DNase I storage solution from -20℃ can be stored at 4 ℃ (for up to 6 weeks). Do not freeze again。

Experiment Preparation - Kit Preparation 2

It is suggested to operate this step in the fume hood

Add β-mercaptoethanol in Buffer RL to make 1% final concentration before operating. For example, add 10 μl β-mercaptoethanol to 1 ml Buffer RL. It is suggested to prepare the lysis buffer right before use. The prepared Buffer RL can be placed in 4℃ for one month. Buffer RL may precipitate during storage. If precipitation occurs, please heat and dissolve before use.

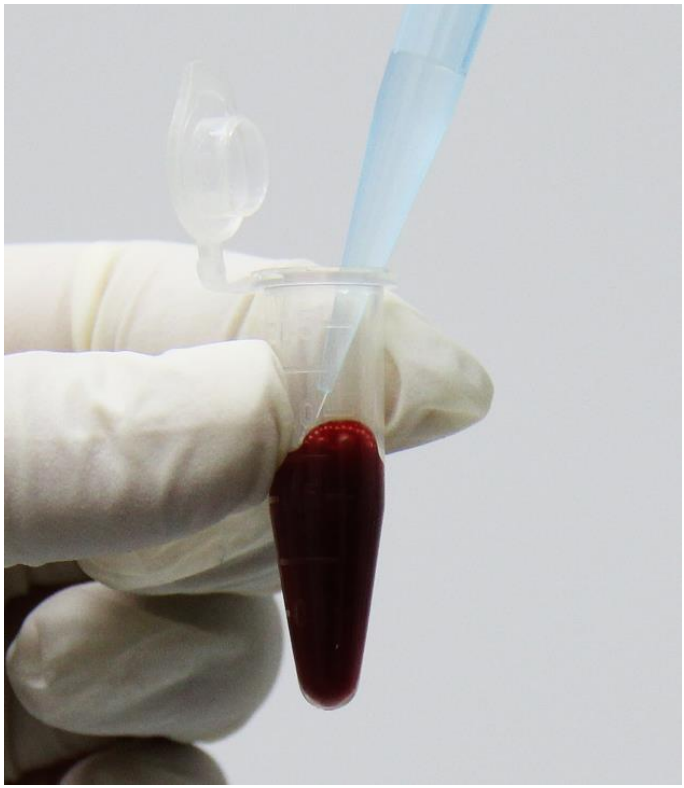
Step 1

Dilution of Red Cell Lysis Buffer

If the volume of the blood sample to be treated is 200 μl, take 140 μl 10× Red Cell Lysis Buffer and dilute with RNase-Free ddH_2O to 1×Red Cell Lysis Buffer.

If multiple samples are extracted at the same time, it is recommended prepare a master DNase I working solution. It is recommended to prepare extra solution to avoid the situation that the total amount is insufficient due to the error of the pipette or the liquid attaching to the tips.

Step 2



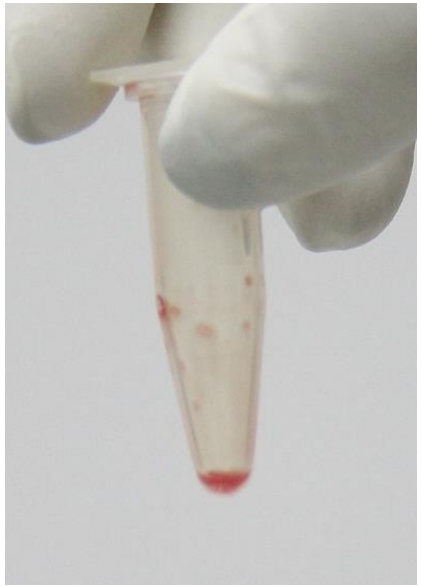
Add 1 ml 1× Red Cell Lysis Buffer to 200 μl human whole blood.

Step 3

Incubate on ice for 10-15 min, during which vortex to mix for two times.

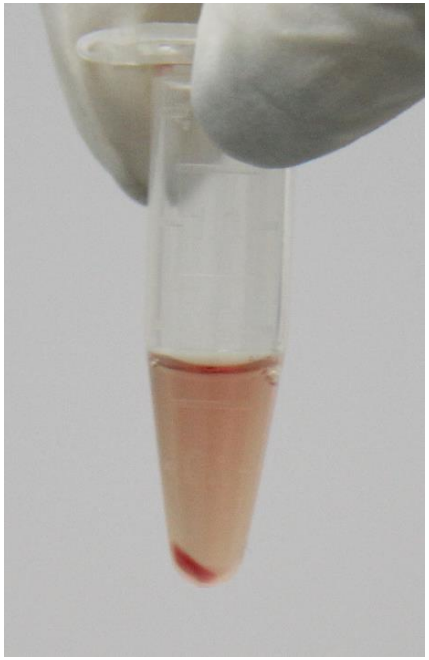
Note: The solution will become translucent during incubation, indicating erythrocyte lysis. Incubation time can be extended to 20 min if necessary.

Step 4



Centrifuge at 2,100 rpm (~400×g) at 4℃ for 10 min, and remove the supernatant completely.

Step 5



Add 1×Red Cell Lysis Buffer to the leukocyte precipitate. (The volume of 1×Red Cell Lysis Buffer added is twice the volume of whole blood used in step 1.)

Repeatedly blow the resuspended cells with a pipette.

Step 6

Centrifuge at 2,100 rpm (~400×g) at 4℃ for 10 min, and remove the supernatant completely.

Step 7

Add Buffer RL to leukocyte precipitate (please add β-mercaptoethanol before use). Please refer to the following table for the specific amount. Mix well by vortex or with a pipette.

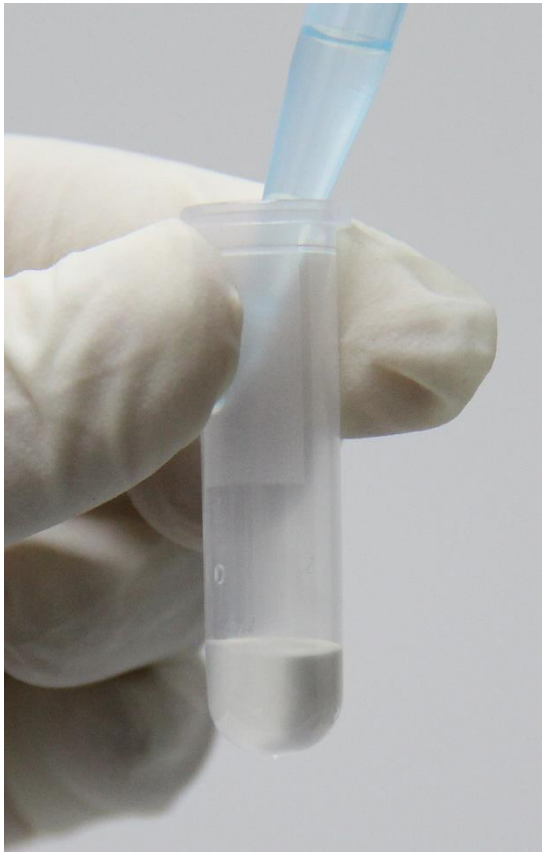
Note: If the blood is not the whole blood of a healthy person, the volume of RL should be determined according to the number of white blood cells in the blood. At this time, the cells should be completely lysed until the massive cell precipitation disappears.

Buffer RL (μl)	Whole blood of healthy person (ml)	Number of leukocyte
350	<0.5	Up to 2×10^6
600	0.5-1.5	2×10^6 to 1×10^7

Step 8

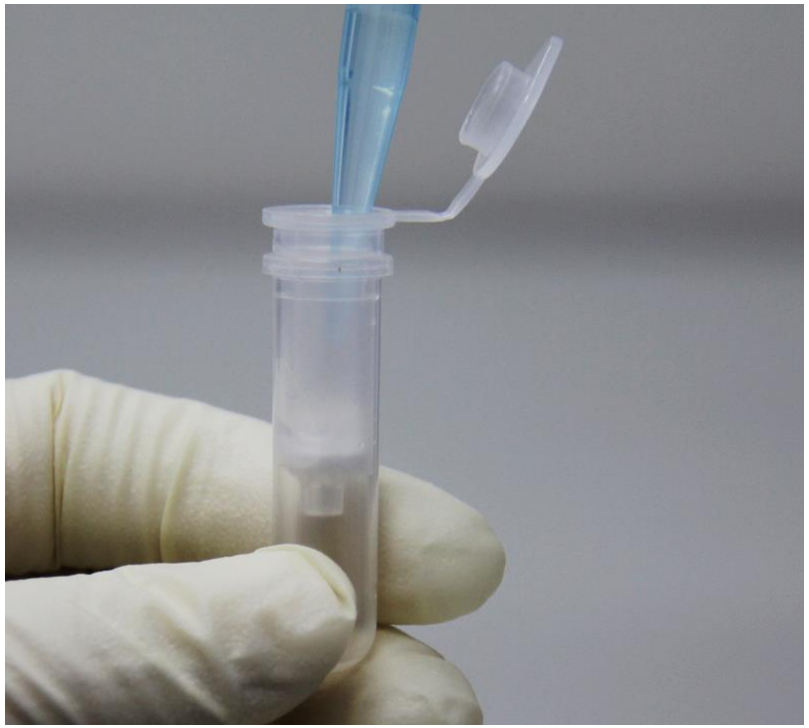
Transfer the solution to the Column CS (place the Column CS in a collection tube), centrifuge at 12,000 rpm (~13,400 g) for 2 min. Discard the Column CS, and collect filtrate.

Step 9



Slowly add 70% ethanol with 1×volume of supernatant, and mix well (precipitation may occur at this time). Transfer the solution and precipitate to a Spin Column CR2. Centrifuge at 12,000 rpm (~13,400×g) for 30-60 sec, discard the waste liquid in the collection tube and put the Spin Column CR2 back into the collection tube.

Step 10



Add 350 μl Buffer RW1 to Spin Column CR2. Centrifuge at 12,000 rpm (~13,400×g) for 30-60 sec, discard the waste liquid and place the Spin Column CR2 into the collection tube.

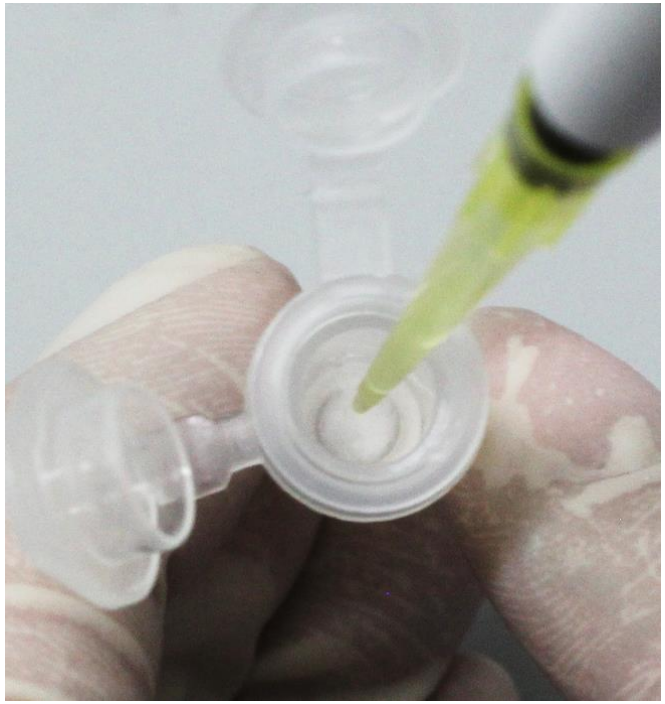
Step 11

Preparation of DNase I working solution:

For 1 sample: Add 10 μl of DNase I storage solution into a new RNase-free centrifuge tube, then add 70 μl Buffer RDD and mix well gently (gently mix with a pipette).

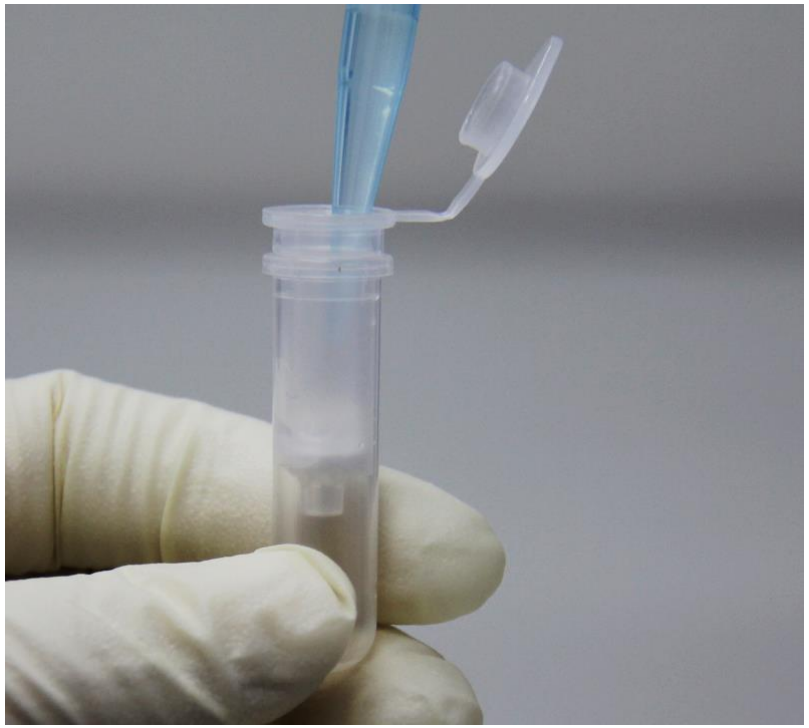
For multiple samples extracted at the same time, please prepare the DNase I working solution together. It is recommended to prepare extra solution to avoid the situation that the total amount is insufficient due to the error of the pipette or the liquid attaching to the tips.

Step 12



Add 80 μl DNase I working solution to the center of Spin Column CR2, and place at room temperature for 15 min.

Step 13



Add 350 µl Buffer RW1 in Spin Column CR2. Centrifuge at 12,000 rpm (~13,400 g) for 30-60 sec, discard the waste liquid, and put the Spin Column CR2 back into the collection tube.

Step 14

Add 500 μl Buffer RW to the Spin Column CR2 (**make sure 96-100% ethanol has been added before use**), place at room temperature for 2 min, and centrifuge at 12,000 rpm (~13,400×g) for 30-60 sec. Pour out the waste liquid and put the Spin Column CR2 back into the collection tube.

Step 15 Repeat step 14.

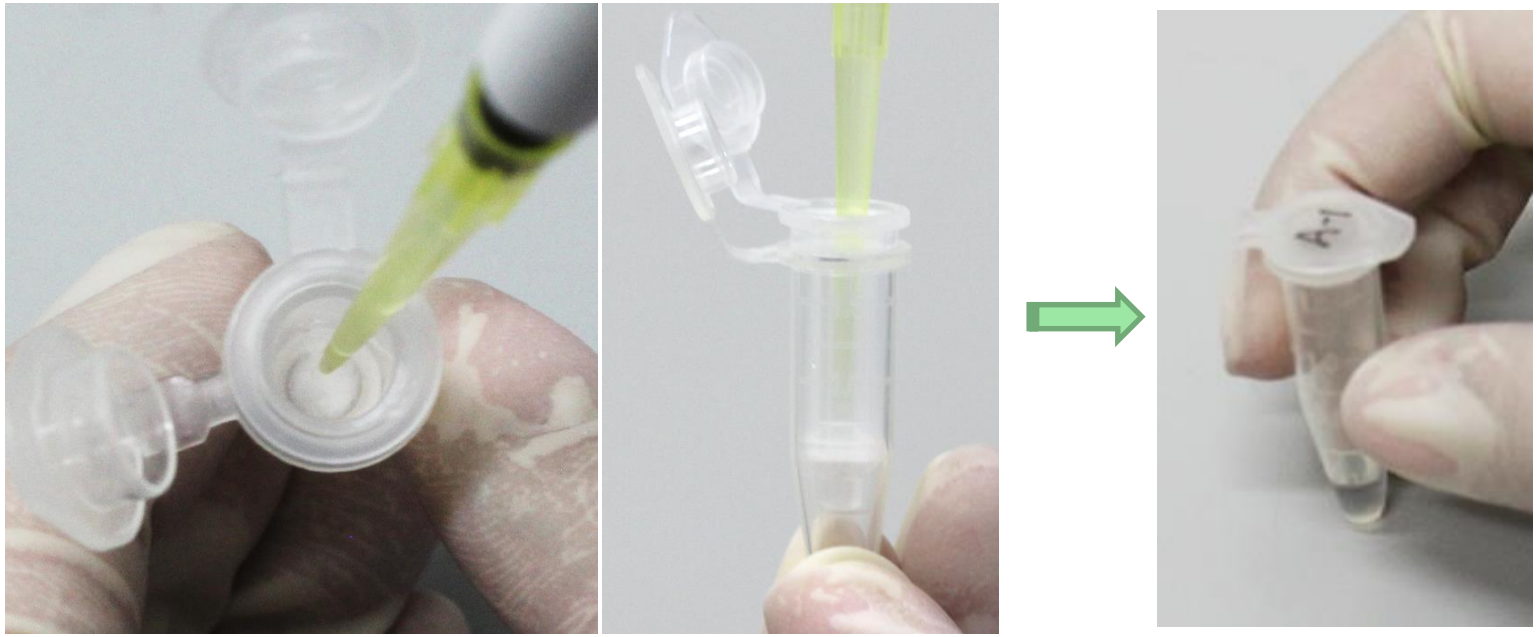
Step 16

Place the Spin Column CR2 in a new 2 ml collection tube. Centrifuge at 12,000 rpm (~13,400×g) for 2 min to remove the residual liquid.

Place the Spin Column CR2 at room temperature for a moment, or place on the clean bench to ventilate for a moment to fully dry the membrane.

Note: Ethanol residues in Buffer RW can inhibit subsequent enzymatic reactions (RT, qPCR, etc.) experiments. However, avoid over-drying, or it will lead to RNA degradation or hard dissolution.

Step 17



Transfer the Spin Column CR2 into the centrifuge tube provided by the kit, add 30–50 μl RNase-Free ddH_2O, place at room temperature for 2 minutes and centrifuge at 12,000 rpm (~13,400×g) for 2 min.

The volume of elution buffer should not be less than 30 μl since too small volume affects the recovery efficiency. RNA should be kept at -70℃ to prevent degradation.