BioChain®
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User's Manual and Instructions

Whole Blood RNA Extraction Kit

Catalog Number: K1012050

Introduction

BioChain's Whole Blood RNA Extraction Kit provides a rapid method for the isolation and purification of RNA molecules from 200µl whole blood samples. The RNA molecules isolated using BioChain's Whole Blood Extraction Kit can be used in various downstream applications relating to gene regulation and functional analysis, including qRT-PCR, northern blotting and microarray profiling analysis.

Features

- **Simple** – quick and easy protocol using the spin-column format, which can isolate blood RNA in less than 30 min.
- **High quality** – isolated RNAs can be used for various downstream applications.
- **Reliable**: repeatable

Applications

- Isolation of RNAs from whole blood.

Description

BioChain's Whole Blood RNA Extraction Kit provides a rapid method for the isolation and purification of RNA molecules from blood. This kit contains enough reagents for 50 isolations of RNA from whole blood.

Column binding capacity	100 µg
Whole Blood	200 µl
Maximum column loading volume	650 µl
Elution Volume	30 – 100 µl

Quality Control

A representative kit from the same lot is randomly selected for isolation of RNAs from whole blood. The quality and purity of isolated small RNAs were measured by denaturing agarose gel electrophoresis and spectrophotometer.

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Kit Components

Item	Part No.	Amount	Storage
1. Lysis Buffer	K1012050-1	33 ml	4°C
2. Lysis Enhancer Solution	K1012050-2	4.4 ml	Room Temp
3. Acidified Phenol:Chloroform	K1012050-3	50 ml	4°C
4. Spin Column with Collection Tubes	K1012050-4	50	Room Temp
5. Wash Buffer 1	K1012050-5	35 ml	Room Temp
6. Wash Buffer 2 (Concentrated)	K1012050-6	7 ml	Room Temp
7. RNase-Free Water	K1012050-7	6 ml	Room Temp

This kit provides enough reagents for 50 isolations of total RNA from 200 µl whole blood.

Items not supplied

1. 100% Ethanol
2. RNase-Free Microcentrifuge Tubes

Storage and Stability

Store the solutions at the appropriate temperature. The kit is stable for one year when handled properly.

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Protocol

I. Things to Do Prior to Use

Add 28 ml of 100% ethanol to the bottle containing the concentrated Wash Buffer 2. Mix well.
This will give a final volume of 35 ml. Mark the bottle to indicate that the ethanol has been added.

II. RNA Isolation Procedure

1. Add 200 µl whole blood sample (fresh or from -80°C) with any anti-coagulant into a new 2 ml tube. (RNA from -80°C blood might be degraded, the kit perform the best for fresh blood)
2. Add 600 µl of Lysis Buffer to the sample. Vortex vigorously until well mixed. (After the blood mix with lysis buffer, you can put the mixture at -20°C for at least one week if you don't have time to process the isolation right away)
3. Add 80 µl of Lysis Enhancer Solution and mix well by shaking vigorously for 15 secs.
4. Add 880 µl (or equal volume) of Acidified Phenol:Chloroform to the above tube and mix well by shaking vigorously for 30 secs. (If no 2 ml tubes are available and start with 1.5 ml tube, Split the solution into another 1.5 ml tube with 440 µl each, and add 440 µl of Acidified Phenol: Chloroform to each tube. (Note: there is water layer in the upper phase, always take Phenol:Chloroform from the lower phase and avoid taking any solution from the upper phase)
5. Centrifuge at 16,000 g (typically ~13,000 rpm at a microcentrifuge) at 4°C for 10 min to separate the aqueous phase (upper phase) and the organic phase (lower phase). Some protein material may be visible at the interphase layer.
6. Carefully remove the upper aqueous phase, ~600 µl in total from the tube(s), containing RNAs to a new RNasefree centrifuge tube. Keep the tube on ice.
7. Add 1 volume of 100% ethanol (~600ul). Mix well by vigorously shaking for 15 sec.
8. Place a spin column in a collection tube. Load 650 µl mixture to the spin column.
9. Close the lid and centrifuge at 10,000 g (~10,000 rpm) at room temperature for 30 sec.
10. Remove the spin column and discard the flow through from the collection tube, then re-seat the spin column in the collection tube.
11. Load the remaining sample to the spin column. Repeat steps 9–10 with the remaining mixture.
12. Add 650 µl Wash Buffer 1 to the spin column and close the lid. Centrifuge at 10,000 g (~10,000 rpm) at room temperature for 30 sec.
13. Discard the flowthrough and repeat step 12 with wash buffer 2.
14. Discard the flow through from the collection tube, then re-seat the spin column in the collection tube. Open the lid and centrifuge at 16,000 g (~13,000 rpm) at room temperature for 1 min.
15. Place the spin column in a new RNase-free microcentrifuge tube; add about 40°C pre-heated 50 – 100 µl RNase-Free Water to the spin column membrane. Close the lid and incubate for 1min. Centrifuge at 16,000 g (~13,000 rpm) at room temperature for 1 min to elute the RNA.

The purified RNA sample may be placed at –20°C for short-term storage or –70°C for long-term storage. (The RNA yield should be from 1- 8 µg from 200 µl blood)