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Protocol Article

One-phase phenol-free method for microRNA isolation from blood plasma

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A B S T R A C T

MicroRNA extraction is an essential procedure when discovering MicroRNA-based biomarkers and approaches. Here we describe a new method for microRNA isolation from human blood plasma, based on isopropanol precipitation from the one-phase lysate. We demonstrate that the use of more than four volumes of lysis buffer based on 5 M guanidine isothiocyanate prevents the formation of large, viscous, and hardly soluble precipitate. Applying widely used linear polyacrylamide (LPAA) as the only precipitating agent proved ineffective. At the same time, adding poly(A)RNA or tRNA with LPAA significantly increased the amount of microRNA obtained. Replacing β-mercaptoethanol with less volatile dithiothreitol in lysis buffer did not lead to a decrease in the yield. We compared the method proposed with miRNeasy Mini Kit (Qiagen) for isolation of microRNA from human blood plasma. MicroRNA yield was evaluated by the difference in median Ct values obtained for exogenous cel-238 and endogenous microRNA-21 cDNA amplification. For both tested microRNA, the precipitation from one-phase lysate provided better recovery with lower Ct values (Δ median Ct 4.94 for cel-238, p = 1,0E-04 and Δ median Ct 2.18 for microRNA-21, p = 9,0E-04). Thus, the method we described showed high yield and operating convenience because it can be applied without special equipment.

A R T I C L E I N F O

Protocol name: One-phase phenol-free method for microRNA isolation from blood plasma
Keywords: Short RNA, Isolation, Biofluids
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Specifications Table

Subject area	Biochemistry, Genetics and Molecular Biology
More specific subject area	MicroRNA isolation
Protocol name	One-phase phenol-free method for microRNA isolation from blood plasma
Reagents/tools	- lysis buffer (5 M guanidine isothiocyanate, 0.75 M NaCl, 0.5 % SDS, 5 % Triton X100, 20 mM TrisHCl, pH = 8) - 1 M DTT - 1 μg/μl poly(A)RNA (or 1 μg/μl yeast tRNA) - 5 μg/μl LPAA - isopropanol - 1 wash buffer (60 % isopropanol, 10 mM TrisHCl, pH = 8.0) - 2 wash buffer (75 % ethanol, 10 mM TrisHC,l pH = 8.0) - RNase-free water
Experimental design	To select the best reagents composition 6 comparative isolations from 8 plasma samples were performed. The following variables were tested: the ratio of the plasma sample volume to the lysis buffer volume, the molarity of NaCl in the lysis buffer, the effect of replacement of β-mercaptoethanol by dithiothreitol and effect of the addition of LPAA and RNA (poly(A)RNA and tRNA) as coprecipitator. As a result, we got the protocol that showed better recovery efficiency: 4 volumes of lysis buffer containing 0.75 M NaCl and adding 2.5 μM DTT, 10 μg poly(A)RNA, 50 μg of LPAA per sample. In comparison with miRNeasy Mini Kit, our protocol showed better yield (Δ median Ct 4.94 for cel-238, p = 1,0E-04 and Δ median Ct 2.18 for microRNA-21, p = 9,0E-04).
Trial registration	–
Ethics	All donors were familiar with the content of the work and signed informed consent.

Value of the Protocol

- The method yields high amounts of high-quality miRNA that is ready for use in any downstream application, including qRT–PCR.
- The method does not use phenol, chloroform, β-mercaptoethanol and other toxic reagents, that should be carefully handled.
- The cost of sample isolation is low.

Description of protocol

MicroRNAs are new potential markers of a wide variety of biological conditions [1,2]. It is a challenge to discover a new markers, and isolation methods have significant role. The method for microRNA isolation from plasma by isopropanol precipitation from one-phase lysate without the use of phenol was proposed and tested. To select the best reagents composition 6 comparative isolations from 8 plasma samples were performed. In comparison with miRNeasy Mini Kit, method proposed showed better yield (Δ median Ct 4.94 for cel-238, p = 1,0E-04 and Δ median Ct 2.18 for microRNA-21, p = 9,0E-04).

Method details

Preparation of plasma

Plasma samples of 8 healthy donors were used. All donors were familiar with the content of the work and signed informed consent. Approximately 10 ml of venous blood was collected into the tube containing EDTA, thoroughly but softly mixed and centrifuged for 10 min at 1600 g at room temperature. The resulting supernatant (approximately 4–5 ml) was carefully selected without capturing the precipitate and transferred into the 15 ml tubes. The supernatant was centrifuged for the second time for 10 min at 1600 g at room temperature and divided into 50 μl aliquots and transferred into 2 ml tubes, frozen and stored at -80 °C for future use.