

Claim

A method for separation of particular types of micro-RNA from biological fluids containing exosomes, comprising sequential centrifugation in 2,000 and 10,000 g mode, ultrafiltration of the resulting supernatant fluid on Centricon plus-70 centrifugal concentrator, 100 kilodaltons, Millipore to a final volume of 10 ml, and ultracentrifugation in Avanti 301 centrifuge (JA-30.50 rotor) in 100,000 g mode for 2 hours. Then the resulting precipitate was dissolved in phosphate-saline buffer and re-centrifuged under the same conditions. Then the resulting precipitate was dissolved in 100 mcl of water, characterized in that further the resulting solution was subjected to free flow electrophoresis in bunkers for anodic and cathodic stabilization at FFE System device followed by collection of the individual fractions under the following conditions: the total time of electrophoresis was 5 minutes, the applied voltage of 900 V and the current of 34 mA. The sample and the marker were introduced at a flow rate of 2.5 ml/h and 280 ml/h, respectively. Running was conducted at a cyclic ZE-FF interval at 85 ml/h and the fractionated sample was eluted at 320 ml/h. The following buffer was used for anodic stabilization: 1,5M of thiourea + 6M of urea + 100 mM of H₂SO₄ + 25 mM of 2-pyridin-propanol + 300 mM of 2-pyridine-ethanol. The medium for separation has the following composition: 1,8 M of thiourea + 6 M of urea + 55 mM of MOPS + 55 mM of 2- pyridine-propanol. The following buffer was used for cathodic stabilization: 1.8 M of thiourea + 76 M of urea + 180 mM of NaOH + 25 mM of Tris + 35 mM of MOPS + 280 mM of EPPS. Then, the separate fractions were re-ultracentrifuged at Avanti 301 centrifuge (JA-30.50 rotor) in 100,000 g mode for 2 h, then micro-RNA is separated from each separate fraction. Namely, 5 uM of proteinase K was added in each separate fraction, incubated at 56 °C for 1 hour, then applied to Exiqon column, followed by centrifugation at 2,000 g, then the column was washed 3 times with wash buffer. After each washing, centrifugation was conducted at 2,000 g at Eppendorf 5104 centrifuge. Then 20 µl of no-nuclease water is applied to the column, then the column is left for 10 minutes at room temperature and then centrifuged at 3,000 g, and then reverse transcription reaction is conducted using reverse transcriptase (Exiqon) namely, 8 µl of the reagent was added to 8 µl of the sample and incubated for 1 hour at 60 °C, after which the reaction was stopped by heating to 95 degrees for 5 minutes.

Claim

- 1) A method for separation of particular types of micro-RNA from biological fluids containing exosomes, comprising sequential centrifugation in 2,000 and 10,000 g mode, ultrafiltration of the resulting supernatant fluid on Centricon plus-70 centrifugal concentrator, 100 kilodaltons, Millipore to a final volume of 10 ml, and ultracentrifugation in Avanti 30I centrifuge (JA-30.50 rotor) in 100,000 g mode for 2 hours. Then the resulting precipitate was dissolved in phosphate-saline buffer and re-centrifuged under the same conditions. Then the resulting precipitate was dissolved in 100 µl of water, characterized in that further the resulting solution was subjected to free flow electrophoresis in bunkers for anodic and cathodic stabilization at FFE System device followed by collection of the individual fractions under the following conditions: the total time of electrophoresis was 5 minutes, the applied voltage of 900 V and the current of 34 mA. The sample and the marker were introduced at a flow rate of 2.5 ml/h and 280 ml/h, respectively. Running was conducted at a cyclic ZE-FF interval at 85 ml/h and the fractionated sample was eluted at 320 ml/h. The following buffer was used for anodic stabilization: 1,5M of thiourea + 6M of urea + 100 mM of H₂SO₄ + 25 mM of 2-pyridin-propanol + 300 mM of 2-pyridine-ethanol. The medium for separation has the following composition: 1,8 M of thiourea + 6 M of urea + 55 mM of MOPS + 55 mM of 2- pyridine-propanol. The following buffer was used for cathodic stabilization: 1.8 M of thiourea + 76 M of urea + 180 mM of NaOH + 25 mM of Tris + 35 mM of MOPS + 280 mM of EPPS. Then, the separate fractions were re-ultracentrifuged at Avanti 30I centrifuge (JA-30.50 rotor) in 100,000 g mode for 2 h, then micro-RNA is separated from each separate fraction. Namely, 5 µM of proteinase K was added in each separate fraction, incubated at 56 °C for 1 hour, then applied to Exiqon column, followed by centrifugation at 2,000 g, then the column was washed 3 times with wash buffer. After each washing, centrifugation was conducted at 2,000 g at Eppendorf 5104 centrifuge. Then 20 µl of no-nuclease water is applied to the column, then the column is left for 10 minutes at room temperature and then centrifuged at 3,000 g, and then reverse transcription reaction is conducted using reverse transcriptase (Exiqon) namely, 8 µl of the reagent was added to 8 µl of the sample and incubated for 1 hour at 60 °C, after which the reaction was stopped by heating to 95 degrees for 5 minutes.