Robotic protocol for RNA extraction by MAVRCIS

Reagents

1. Binding buffer:
    - 10 mM Bis-Tris /HCl pH< 6.5, 3M guanidium hydrochloride, in 90% Ethanol
2. SiMNP in 96-well plate
3. Wash buffer:
    - Binding buffer + TRIzol at 1:1 v/v
4. Ethanol wash
    - 90% Ethanol

Materials

1. Magnet for bead separation in plates
    - V&P Scientific, Inc. Cat. VP 771MDWZM-1-ALT
2. Patient Sample plate (96 deepwell)
    - Any deepwell plate
    - Same plates can be used for 2 waste positions
3. Extraction-plate (96 deepwell, square-well, round-bottom)
    - Whatman, Cat. 7701-5200
4. Microplate for bead input (regular 96-well, round-bottom)
    - e.g. Corning Cat. 3788
5. Universal microplate lids
    - Whatman / Cytivia Cat. 77041001
    - Fit all the plates used
6. Evo MCA96 nested tips 200µl sterile
    - Axygen, Cat. EV-200-NTR-S

Notes

1. Trizol contains phenol and guanidinium isothiocyanate and needs to be discarded accordingly.

TECAN EVO Script Summary

- volumes are per well
- all plates (except 2nd waste position) are covered with lids, to be removed before each pipetting step and placed back afterwards

RNA Binding	Pickup MCA tips
	Add 2 x 150µl binding buffer to extraction plate
	Briefly shake microplate containing beads (resuspend)
	Transfer 40µl beads solution to extraction plate (pipette up / down on aspiration)
	Transfer 2 x 150µl patient sample from input plate to extraction plate
	Shake 5 min @ 1300 rpm
	Move extraction plate to magnet, 30s – 1 min settling time
	Aspirate and discard supernatant
	Move extraction plate back to regular pipetting position
	Change MCA tips
Wash I	Add 2 x 150µl wash buffer
	Shake 20s @ 1300 rpm
	Move extraction plate to magnet, 30s – 1 min settling time
	Aspirate and discard supernatant
	Move extraction plate back to regular pipetting position
	Change MCA tips
Ethanol Wash ***repeat 4x***	Add 2 x 150µl 90% EtOH
	Shake 20s @ 1300 rpm
	Move extraction plate to magnet, 30s – 1 min settling time
	Aspirate and discard supernatant
	Move extraction plate back to regular pipetting position
	Change MCA tips (optional between steps)
Drying	Move extraction plate to heat block, incubate 20 min @ 50 C
	Move extraction plate back to regular pipetting position
Elution	Add 40µl nuclease-free water to extraction plate wells
	Shake 5 min @ 1300 rpm
	Move extraction plate to magnet, 30s – 1 min settling time
	Move destination plate from hotel to pipetting position, remove lid
	Transfer supernatant from extraction plate to destination plate, place back lid
	Discard tips