# 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

|  |  |
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|  |  |
|  | Pickup MCA tips |
| RNA Binding | 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 | Add 2 x 150µl 90% EtOH |
| Wash | Shake 20s @ 1300 rpm |
| **repeat 4x** | 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 |