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Magnetic Bead DNA/RNA extraction using the NucleoMag DNA/RNA Water kit and the 96 manual magnetic bead extractor – 96 well format

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We use this protocol and it's working

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Abstract

Protocol for magnetic Bead DNA/RNA extraction using the NucleoMag DNA/RNA Water kit and the 96 manual magnetic bead extractor – 96 well format

Materials

- NucleoMag DNA/RNA Water kit (Machery Nagel)

- 1× 96 Magnet bead extractor pin tool (V&P Scientific)

- ***1x rDNAse set + extra rDNA buffer (Machery Nagel) (only for RNA extraction)***

- 4× 96 well square blocks, small size ***(one more for RNA extraction)***

- 1× 96 well plate, 300 µL (circa)

- 1x Non-skirted PCR plate to cover magnet

- ***1× 50 mL falcon tube (for rDNAse mix) (only for RNA extraction)***

- 2x high racks to make a “drying rack” for air drying

- 5x Reagent matrices (25mL) ***(two more for RNA extraction)***

- 6x plate cover foil ***(one more for RNA extraction)***

- 1× 8-channel 100-1000 Rainin pipette

- 1× 12-channel 20-200 Rainin pipette

Troubleshooting

Before start

*Note: for wells 2-5(DNA)/**6(RNA)**, transfer volume needed for 96 samples into reagent matrices, pipette volumes into the wells using a multichannel pipette, and cover all wells with a plate cover foil immediately after preparation*

- Clean a hood with bleach + EtOH + Eliminase, number wells from 1-5(6), UV all tools, wells, racks, tubes, and reagent matrices

- rDNAse mix in a 50 mL falcon tube according to manual (37.5 µl rDNAse + 262.5 µl rDNA buffer per sample -> for 96 samples: 28 mL rDNAse buffer plus rDNAse reconstituted in 4 mL buffer = 32 mL) (only for RNA extraction)

- 1. well (square): 200 µl lysate per sample and 200 µl RNAse-free water for negative controls (***prepare this well last***; when preparing, start at the top row of the well and cover empty rows with a plate foil cover, then work your way down; that way, you avoid going over open wells with tips that contain lysate (potential cross-contamination))

a.If samples are already in a 96-well plate: transfer the lysate from the plate to the well using a multichannel pipette; make sure to follow a premade template with 1 negative control per row (adding up to 12 negative controls)

b.If samples are in tubes: transfer samples to PCR 12-strip tubes following a premade template, add negative controls accordingly (one per row), just prep one row at a time and transfer the lysate using a multichannel pipette

- 2. well (square): 650 µl buffer MWA3 per sample (*note: protocol says 850 µl but well would overflow*) (x 96 = 62.4 mL; when prepping fill reagent matrix to 25 mL, then refill to 25 mL, then refill half)

- 3. well (square): 650 µl buffer MWA3 per sample (x 96 = 62.4 mL; when prepping, fill the reagent matrix to 25 mL, then refill to 25 mL, then refill half)

- 4. well (square): 650 µl buffer MWA4 per sample (x 96 = 62.4 mL; when prepping, fill the reagent matrix to 25 mL, then refill to 25 mL, then refill half)

- 5. well (300µl well): 100 µl water per sample (x 96 = 9.6 mL; when prepping, fill 10 mL into reagent matrix)

- 6. well (square): 300 µl rDNAse mix per sample (x 96 = 28.8 mL; when prepping, fill the reagent matrix to 25 mL, then refill the rest of the rDNA mix into the same reagent matrix)

1 Add 25 µl B-beads to each lysate in well 1 with a multichannel pipette and cover all other rows while pipetting into one row → for 96 samples + 2 buffer volumes, fill 2,450 µl B-beads into a reagent matrix

2 Add 425 µl MWA2 buffer to each sample with a multichannel pipette (note: protocol says 475 µl buffer MWA2 but wells would overflow), cover all other rows while pipetting into one row → for 96 samples + 2 buffer volumes, fill 41.65 mL MWA2 buffer into a reagent matrix; mix samples, buffer, and beads in well by pipetting 10x up and down after transferring the buffer to the samples

3 Wait for 5 min (nucleic acids bind to beads)

4 Hold the magnet into well 1, move it around for 30 secs (to catch all magnetic beads (probably unnecessary but it made me feel better))

5 Move the magnet into well 2, DON'T discharge the cover plate (beads will continue to stick to magnets and buffers will interact with beads on the magnet), wait for 3 min

6 Move the magnet into well 3, wait for 3 for min

7 If RNA extraction:

7.1 Move the magnet out of well 3, air dry beads for 5 min

7.2 Move the magnet to well 6, wait for 25 min (nucleic acids unbind from magnetic beads and go into solution, DNA is digested) (note: the protocol says to wait for 15 min but I still had DNA in my samples after 15 min)

7.3 Take the magnet including the magnetic beads out and set them aside on the drying rack

7.4 Add 350 µl buffer MWA2 to well 6, cover all other rows while pipetting into one row, mix by pipetting 10x up and down, wait for 5 min (preparing nucleic acids to rebind) (350 µl x 96 = 33.6 mL, use the same technique for this step as when prepping the wells: fill up the reagent matrix to 25 mL, use up the mix, then refill to same reagent matrix to 1/3rd)

7.5 Move the magnet including the magnetic beads back in well 6, wait for 5 min (nucleic acids re-bind to beads)

8 Move the magnet into well 4, wait for 3 min

9 Move the magnet out of well 4, air dry beads for 10 min

10 Move the magnet into well 5, wait for 10 min (nucleic acids unbind from magnetic beads and go into final elution)

11 Cover the plate containing the eluates with foil and store it in a -80 freezer (-20 is sufficient for DNA samples)