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Integra Total Nucleic Acid Extraction

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Protocol status: Working
We use this protocol and it's working

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Abstract

This SOP is the process of extracting Total Nucleic Acid (TNA) from Sera and/or Nasopharyngeal or Nasal swabs. The isolated high-quality nucleic acids are suitable for Next-Generation Sequencing (NGS).

Guidelines

Adapted from the Quick-DNA/RNA Pathogen MagBead Kit Manual (Zymo Research, Cat# R2145)

Materials

1. RNase away sprays for RNase decontaminants (Thermo Scientific, Cat#7002).

RNase Away spray **Molecular BioProducts**

2. 1ml deep well sterile plate.

3. 2ml deep well sterile plate.

4. Hard-shell PCR Plates 96-well (Bio-Rad, Cat# HSP9601).

5. PCR Plate Seal, foil (Bio-Rad, Cat# MSF1001).

6. Quick-DNA/RNA Pathogen MagBead kit (Zymo Research, Cat# R2145)

Quick-DNA/RNA Pathogen MagBead kit **Zymo Research Catalog #**R2145

7. Molecular-Grade Isopropanol (100% isopropanol).

8. Molecular-Grade Absolute ethanol (100% ethanol).

9. Proteinase K w/ Storage buffer 20mg (Zymo Research, Cat# D3001-2-20).

Proteinase K w/ Storage buffer 20mg set **Zymo Research Catalog #**D3001-2-20

10. DNase/RNase-free water

Nuclease-free water **Ambion Catalog #**AM9932

11.

Equipment

VIAFLO	NAME
96 channel pipette	TYPE
Integra	BRAND
VIAFLO 96	SKU
https://www.integra-biosciences.com/united-kingdom/en/electronic-pipettes/viaflo-96-viaflo-384#tech-info	LINK

12. DNase I (Zymo Research, Cat# E1010)

DNase I set **Zymo Research Catalog #**E1010

13. 96-well magnetic stand

Safety warnings

All steps should be performed at Room temperature .

*__NOTE__: When reusing tips, make sure to include a bit of extra air aspiration to avoid drops at the bottom of tips when aspirating volumes, and also a bit extra air blows out at the end of dispensing steps in plates.

Perform the TNA extraction in the extraction room separate from the PCR room.
Respect the Laboratory safety guideline for all steps of the protocol.
Wearing PPE is recommended.

Reagent preparation (required with new kit)

15m

1

15m

1. Add 20 mL of isopropanol to the MagBead DNA/RNA Wash 1 concentrate.
2. Add 30 mL of isopropanol to the MagBead DNA/RNA Wash 2 concentrate.
3. Add 1.2 mL of Proteinase K Storage Buffer per vial to reconstitute the lyophilized Proteinase K at [M] 20 mg/mL

Vortex to dissolve. STORE AT -20 °C Freezer.

Preparation the buffer plate (before starting protocol)

1h

2

1h

1. Pre-make pathogen buffer plate with 880 µL Pathogen DNA/RNA buffer in 1ml deep well plate.
2. Pre-make bead plate with 25 µL MagBinding beads into 96V-well PCR plate.

*Make immediately before starting, <1h prior to starting the protocol, to ensure the beads are mixed.

3. Pre-make DNA/RNA Wash 1 plate with 550 µL Wash 1 buffer into a 1ml deep well plate.
4. Pre-make DNA/RNA Wash 2 plate with 550 µL Wash 2 buffer into a 1ml deep well plate.
5. Pre-make 100% ethanol plate with 1 mL 100% ethanol into a 2ml deep well plate.
6. Pre-make 80% ethanol plate with 600 µL 80% ethanol into a 1ml deep well plate.
7. Pre-make water plate with 50 µL DNAase/RNAse-free water in a 96 V-well PCR plate.
8. Pre-make water plate with 30 µL DNAase/RNAse-free water in a 96 V-well PCR plate.

Spin all plates down for 00:01:00 except the bead plate. Perform a quick pulse spin down of the bead plate, just enough to get all liquid down. Centrifuge the rest of the plates at 12 000 rpm for 00:01:00 .

Sample preparation and Proteinase K

16m

3

16m

1. Create a plate map so you know which sample you are adding to each well. Add 400 µL of your samples to 2ml deep well plate (Plate 1).

2. Manually add 65 µL of Proteinase K to each well of 8 well PCR strip tubes. Using a manual multichannel pipet, aliquot 4 µL of Proteinase K into each sample (Plate 1).
3. Load a set of Integra tips (tip set 1) onto the Integra.
4. **Program: Pipet/Mix 250ul, 15 cycles, speed 10.** Program the Integra to pipet 250 µL of your samples up and down for 00:01:00 (15 cycles), then incubate at Room temperature for 00:15:00 .
*Keep tips.

Sample binding and washing

1h 20m

4

1h 20m

5. **Program: Pipet 300ul.** add 800 µL total of Pathogen DNA/RNA Buffer to the sample plate (Plate 1).
6. **Program: Pipet/Mix 250ul, 30 cycles, speed 10.** Program the Integra to mix samples and buffer for 00:02:00 . Keep tips.
7. **Program: Pipet/Mix 20ul, 20 cycles.** Program the Integra to mix the MagBinding beads plate so the beads are fully resuspended. If beads are not fully to the bottom of theplate, perform a short pulse spin.
8. **Program: Pipet 20ul.** Program the Integra to aspirate 20 µL from the MagBinding beads plate. Check that all wells have beads!
9. **Program: Pipet/Mix 250ul, 30 cycles x 5 (10 min total), speed 7.** Program the Integra to mix the beads with the sample for 00:10:00 total. Keep tips.
10. Place the 96-well magnetic stand underneath the sample plate for 00:05:00 until a bead ring forms.
11. **Program: Manual Pipet 300ul.** Aspirate and discard the cleared supernatant into a 2ml deep well waste plate.
Discard tips, load new tips.
12. Remove magnetic stand.
13. **Program: Pipet 250ul.** Dispense a total of 500 µL Wash 1 into the sample plate. Keep tips.
14. **Program: Pipet/Mix 250ul, 30 cycles, speed 7.** Program the Integra to mix the beads with the Wash buffer for 00:02:00 total. Keep tips.
15. Place the 96-well magnetic stand underneath the sample plate for 00:02:00 until bead ring forms.
16. **Program: Manual Pipet 300ul.** Aspirate and discard the cleared supernatant into the 2ml deep well waste plate. Keep tips.
17. **Program: Pipet 250ul.** Dispense a total of 500 µL Wash 2 into the sample plate. Keep tips.

18. **Program: Pipet/Mix 250ul, 30 cycles, speed 7.** Program the Integra to mix the beads with the wash buffer for 00:02:00 total. Keep tips.
19. Place the 96-well magnetic stand underneath the sample plate for 00:02:00 until a bead ring forms.
20. **Program: Manual Pipet 300ul.** Aspirate and discard the cleared supernatant into the 2ml deep well waste plate. Discard tips, load new tips.
21. Remove magnetic stand.
22. **Program: Pipet 250ul.** Dispense a total of 500 µL 100% Ethanol into the sample plate.
23. **Program: Pipet/Mix 250ul, 30 cycles, speed 7.** Program the Integra to mix the beads with Ethanol for 00:02:00 total. Keep tips.
24. Place the 96-well magnetic stand underneath the sample plate for 00:02:00 until a bead ring forms.
25. **Program: Manual Pipet 300ul.** Aspirate and discard the cleared supernatant into the 2ml deep well waste plate.
26. Remove magnetic stand.
27. Repeat steps 22 to 26 for another round of Ethanol wash. Discard tips after 2nd Ethanol wash. Load new tips.
28. Keep the sample plate on the magnetic stand after the final Ethanol wash until the beads are dry (~ 00:10:00).
29. **Program: Pipet/Mix 33ul, 30 cycles, speed 7.** Pipet 33 µL of Nuclease-free water (Heat the water at 55 °C for 00:10:00 before elution) into the dried beads and mix the beads for 00:02:00 total. Keep tips.
30. Place the 96-well magnetic stand underneath the sample plate for 00:02:00 until a bead ring forms.
31. **Program: Manual Pipet 30ul.** Pipet 30 µL from the sample plate and dispense into a new 96 V-bottom PCR plate.
32. Store TNA sample immediately at -80 °C .

DNase treatment post-TNA purification

15m

5

1. Aliquot SPRI beads (well mixed and resuspended) 50 µL into a new 96 V-bottom PCR plate.

15m

2. Prepare DNase I solution
   a. Add 275 µL DNase/RNase-free water to reconstitute lyophilized DNase I and mix.

3. Aliquot 20 µL of TNA into a new 96 V-bottom PCR plate.
4. Prepare DNase master mix:
   a. 1x rxn = 20ul sample + 2.5ul DNase I + 2.5ul Digestion Buffer.
   b. Using a manual multichannel pipet, aliquot 5 µL of DNase I master mix to each sample and mix.
   c. **Program: Pipet/Mix 20ul, 20 cycles, speed 7.**
5. Incubate 00:15:00 Room temperature

SPRI bead clean-up

30m

6

30m

1. **Program: Pipet/Mix 45ul, 20 cycles, speed 7.** Add 45 µL SPRI beads (1.8x ratio) to the DNase I treated sample and mix by pipetting.
2. Incubate 00:05:00 at Room temperature .
3. Place the 96-well magnetic stand underneath the sample plate for 00:02:00 until a bead ring forms.
4. **Program: Manual Pipet 100ul.** Aspirate and discard the cleared supernatant into the 2ml deep well waste plate. Keep tips.
5. **Program: Pipet 200ul. (1st Ethanol Wash).** Aspirate and dispense 200 µL of freshly made 80% Ethanol into sample plate.
6. Incubate for 00:01:00 at Room temperature .
7. **Program: Manual Pipet 200ul.** Aspirate and discard the cleared supernatant into the 2ml deep well waste plate. Keep tips.
8. **Program: Pipet 200ul. (2nd Ethanol Wash).** Aspirate and dispense 200 µL of freshly made 80% Ethanol into sample plate.
9. Incubate for 00:01:00 at Room temperature .
10. **Program: Manual Pipet 200ul.** Aspirate and discard the cleared supernatant into the 2ml deep well waste plate. Discard tips, load new tips.
11. Incubate for 00:05:00 at Room temperature until beads are dry.
12. Remove plate from magnetic stand.
13. **Program: Pipet/Mix 22ul, 10 cycles, speed 7.** Add 22 µL DNase/RNase-free water to each well and mix beads.
14. Place the 96-well magnetic stand underneath the sample plate for 00:02:00 until a bead ring forms.
15. **Program: Manual Pipet 20ul.** Aspirate 20 µL of purified TNA sample to a new 96 V-bottom PCR plate.
16. Store sample at -80 °C immediately.