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Integra Magbead DNA and RNA Extraction for isolated colonies

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We use this protocol and it's working

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Abstract

This protocol is the process to extract DNA and RNA from isolated colonies. The extracted high-quality DNA or RNA are suitable for Next-Generation Sequencing (NGS).

Guidelines

Adapted from the ZymoBIOMICS MagBead DNA/RNA Kit Manual (Zymo Research, Cat#R2135).

Materials

1. RNase away spray for RNase decontaminants.
RNase AWAY™ Surface Decontaminant **Thermo Fisher Scientific Catalog #**7002PK

2. ZymoBIOMIC MagBead DNA/RNA **Zymo Research Catalog #**R2135
3. 100% Molecular grade ethanol
4. Molecular Grade Isopropanol
5. Proteinase K w/ Storage buffer 20mg set **Zymo Research Catalog #**D3001-2-20
6. DNase I Set **Zymo Research Catalog #**E1010
7. Nuclease-free water **Ambion Catalog #**AM9932

8. 1ml deep well sterile plate.

9. 2ml deep well sterile plate.

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

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

12. 96S Super Magnet. (ALPAQUA, Cat# A001322)

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

Troubleshooting

Safety warnings

All steps should be performed at Room temperature .

Perform the 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.

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 of extra air blows out at the end of dispensing steps in plates.

Buffer Preparation

30m

1

30m

1. Add 20 mL isopropanol to the MagBead DNA/RNA Wash 1 concentrate.
2. Add 30 mL isopropanol to the MagBead DNA/RNA Wash 2 concentrate.
3. Reconstitute lyophilized Proteinase K at [M] 20 mg/mL with Proteinase K Storage Buffer and mix by vortexing. Use immediately or store at -20 °C .
4. Reconstitute each vial of lyophilized DNase I with 2.25 mL DNase/RNase-Free water in a conical tube.

Note

For each sample to be treated, prepare DNase I Reaction Mix (scale up proportionally):
Add 45 µL DNase I (reconstituted) and 5 µL DNA Digestion Buffer in a nuclease-free tube.
mix by gentle inversion and place On ice until ready to use.

Make buffer plates prior to starting protocol

1h

2

1h

1. Pre-make Lysis Buffer plate with 520 µL DNA/RNA Lysis buffer in 1ml deep well plate.
2. Pre-make Beads plate with 35 µL ZymoBIOMIC MagBinding Beads into 96 V-well PCR plate.

Note

For the Beads plate, make it immediately before starting, <1h prior to starting the protocol, to ensure the beads are kept in suspension.

3. Pre-make DNA/RNA Wash 1 plate with 520 µL MagBead DNA/RNA Wash 1 into 1ml deep well plate. Make it two plates.
4. Pre-make DNA/RNA Wash 2 plate with 520 µL MagBead DNA/RNA Wash 2 into 1ml deep well plate. Make it two plates.

5. Pre-make 100% Ethanol plate with 1100 µL of 100% Ethanol into a 2ml deep well plate. Make it three plates.
6. Pre-make Prep Buffer plate with 520 µL DNA/RNA Prep Buffer into a 1ml deep well plate.
7. Pre-make water plate with 60 µL Nuclease-free water in a 96 V-well PCR plate. Make it two plates.
8. Spin all plates down for 00:01:00 except for the bead plate. Perform a quick pulse spin down of the bead plate, just enough to get all the liquid down. Centrifuge the rest of the plate at 12 000 rpm for 00:01:00 .

Sample preparation and Proteinase K

31m

3

31m

1. Create a plate map so you know which sample you are adding to each well. Add 50 µL of isolated colonies samples to plate 1 (leave column 12 for water control).
2. Top up the 1x DNA/RNA Shield to get 750 µL .
3. Manually add 120 µL of Proteinase K into the 0.2ml 8-strip well.
4. Use multichannel pipet to add 10 µL of Proteinase K into each sample and mix (plate 1).
5. Load a set of Integra tips (tip set 1) onto the Integra.
6. **Program: Pipet/Mix 250ul, 15 cycles, speed 4.** 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 00:30:00 . Keep tips.

Sample binding and washing

35m

4

35m

7. **Program: Pipet 250ul.** Add 500 µL total of Lysis Buffer to the sample plate (plate 1).
8. **Program: Pipet/Mix 250ul, 30 cycles, speed 10.** Program the Integra to mix samples and buffer for 00:02:00 . Keep tips.
9. Aliquot 35 µL of MagBinding Beads into 96 V-well PCR plate.
10. **Program: Pipet/Mix 20ul, 10 cycles, 2 times, speed 4.** Program the Integra to mix the MagBinding Beads plate, so the beads are fully resuspended.
11. **Program: Pipet 30ul.** Add 30 µL of MagBinding Beads into the sample plate (plate 1).

12. **Program: Pipet/Mix 250ul, 30 cycles, speed 3.** Program the Integra to mix the sample and MagBinding Beads plate, so the beads are fully resuspended. Continue this Integra Program to mix the sample and MagBinding Beads for 00:20:00 .

13. Transfer the plate/tube to the magnetic stand for 00:05:00 until beads (DNA) have pelleted, transfer the cleared supernatant (RNA) into a new 96 V-well plate.

DNA Purification (Beads)

45m

5

45m

14. Change new Integra tips.

15. **Program: Pipet 250ul, 2 times, speed 7.** Dispense a total of 500 µL MagBead DNA/RNA Wash 1 into sample plate and mix well.

16. **Program: Pipet/Mix 250ul, 30 cycles, speed 10.** Program the Integra to mix the Wash 1 buffer with the beads. Keep tips.

17. Place the 96-well magnetic stand underneath the sample plate for 00:02:00 until a bead ring forms.

18. **Program: Manual Pipet 250ul, 2 times, speed 3.** Aspirate and discard the cleared supernatant into a 2ml deep well waste plate.

19. **Program: Pipet 250ul, 2 times, speed 7.** Dispense a total of 500 µL MagBead DNA/RNA Wash 2 into sample plate and mix well.

20. **Program: Pipet/Mix 250ul, 30 cycles, speed 10.** Program the Integra to mix the Wash 2 buffer with the beads. Keep tips.

21. Place the 96-well magnetic stand underneath the sample plate for 00:02:00 until a bead ring forms.

22. **Program: Manual Pipet 250ul, 2 times, speed 3.** Aspirate and discard the cleared supernatant into a 2ml deep well waste plate.

23. Change new Integra tips.

24. **Program: Pipet 250ul, 2 times, speed 7.** Dispense a total of 500 µL 100% Ethanol into sample plate and mix well.

25. **Program: Pipet/Mix 250ul, 30 cycles, speed 10.** Program the Integra to mix 100% Ethanol with the beads. Keep tips.

26. Place the 96-well magnetic stand underneath the sample plate for 00:02:00 until a bead ring forms.

27. **Program: Manual Pipet 250ul, 2 times, speed 3.** Aspirate and discard the cleared supernatant into a 2ml deep well waste plate.

28. Repeat step 24.

29. Dry the beads for 00:10:00 on the magnetic stand.

30. Change new Integra tips.

31. **Program: Pipet 30ul, speed 5.** Dispense a total of 30 µL nuclease-free water into the sample plate.
32. **Program: Pipet/Mix 20ul, 30 cycles, speed 7.** Program the Integra to mix nuclease-free water with the beads. Keep tips.
33. **Program: Manual Pipet 30ul, speed 3.** Transfer the plate to the magnetic stand and pellet the beads for 00:05:00 , then aspirate and dispense the eluted DNA to a new 96 V-well plate.
34. Store DNA sample immediately at -80 °C .

RNA Purification (Supernatant) 45m

6 35. Change the new Integra tip. 45m

36. **Program: Pipet 230ul, 3 times, speed 7.** Dispense a total of 690 µL 100% Ethanol to the supernatant.
37. **Program: Pipet/Mix 250ul, 30 cycles, speed 7.** Program the Integra to mix 100% Ethanol with the supernatant. Keep tips.
38. Aliquot 35 µL of MagBinding Beads into 96 V-well PCR plate.
39. **Program: Pipet/Mix 20ul, 10 cycles, 2 times, speed 4.** Program the Integra to mix the MagBinding Beads plate, so the beads are fully resuspended.
40. **Program: Pipet 30ul.** Add 30 µL of MagBinding beads into the sample plate.
41. **Program: Pipet/Mix 250ul, 10 cycles, speed 3.** Program the Integra to mix the sample and MagBinding beads
plate, so the beads are fully resuspended. Continue this Integra Program to mix the sample and MagBinding Beads for 00:10:00 .
42. Transfer the plate to the magnetic stand for 00:05:00 until beads have pelleted, then discard the cleared supernatant.
43. **Program: Pipet 250ul, 2 times, speed 7.** Dispense a total of 500 µL MagBead DNA/RNA Wash 1 into sample plate.
44. **Program: Pipet/Mix 250ul, 30 cycles, speed 10.** Program the Integra to mix the Wash 1 buffer with the beads. Keep tips.
45. Place the 96-well magnetic stand underneath the sample plate for 00:02:00 until a bead ring forms.
46. **Program: Manual Pipet 250ul, 2 times, speed 3.** Aspirate and discard the cleared supernatant into a 2ml deep well waste plate.
47. **Program: Pipet 250ul, 2 times, speed 7.** Dispense a total of 500 µL MagBead DNA/RNA Wash 2 into sample plate.

48. **Program: Pipet/Mix 250ul, 30 cycles, speed 10.** Program the Integra to mix the Wash 2 buffer with the beads. Keep tips.
49. Place the 96-well magnetic stand underneath the sample plate for 00:02:00 until a bead ring forms.
50. **Program: Manual Pipet 250ul, 2 times, speed 3.** Aspirate and discard the cleared supernatant into a 2ml deep well waste plate.
51. **Program: Pipet 250ul, 2 times, speed 7.** Dispense a total of 500 µL 100% Ethanol into the sample plate.
52. **Program: Pipet/Mix 250ul, 30 cycles, speed 10.** Program the Integra to mix 100% Ethanol with the beads. Keep tips.
53. Place the 96-well magnetic stand underneath the sample plate for 00:02:00 until a bead ring forms.
54. **Program: Manual Pipet 250ul, 2 times, speed 3.** Aspirate and discard the cleared supernatant into a 2ml deep well waste plate.
55. Repeat step 51.
56. **DNase I** treatment, use multiple channel pipet to transfer 50 µL of DNase I Reaction Mix and mix gently for 00:10:00 .
57. **Program: Pipet 250ul, 2 times, speed 7.** Dispense a total of 500 µL DNA/RNA Prep Buffer into sample plate.
58. **Program: Pipet/Mix 250ul, 30 cycles, speed 10.** Program the Integra to mix the DNA/RNA Prep Buffer with the beads. Keep tips.
59. Place the 96-well magnetic stand underneath the sample plate for 00:02:00 until a bead ring forms.
60. **Program: Manual Pipet 250ul, 2 times, speed 3.** Aspirate and discard the cleared supernatant into a 2ml deep well waste plate.
61. Repeat step 57 to 60.
62. **Program: Pipet 30ul, speed 5.** Dispense a total of 30 µL nuclease-free water into the sample plate.
63. **Program: Pipet/Mix 20ul, 30 cycles, speed 7.** Program the Integra to mix nuclease-free water with the beads. Keep tips.
64. **Program: Manual Pipet 30ul, speed 3.** Transfer the plate to the magnetic stand and pellet the beads for 00:05:00 , then aspirate and dispense the eluted RNA to a new 96 V-well plate.
65. Store RNA sample immediately at -80 °C .