Axygen® AxyPrep MAG Viral Nucleic Acid Purification Kit

High throughput purification of viral nucleic acid (RNA and DNA) from biofluids

Protocol

Introduction

The AxyPrep MAG Viral Nucleic Acid Purification Kit is designed for scalable, rapid, and reliable isolation of high quality total nucleic acid (RNA and DNA) from a broad range of sample materials, including: whole blood, serum, plasma, saliva, tongue scrapings, throat wash samples, and other bodily fluids, or transport media.

High quality RNA and DNA is suitable for direct use in most downstream applications such as sequencing, enzymatic reactions, and real-time PCR assays. The kit can be adapted to most major automated magnetic bead separation instruments and workstations in the market.

Process

Samples are lysed in a specially formulated buffer containing detergent. Nucleic acid is bound to the surface of MAG-S1 particles under proper conditions. Proteins and cellular debris are efficiently washed with few wash steps. Purified RNA and DNA are then eluted in nuclease-free water or low ionic strength buffer. Purified RNA or DNA can be directly used in downstream applications without the need for further purification.

Kit Contents and Storage

	Cat. No.		
	MAG-VNA-S	MAG-VNA-M	Storage
Number of Preps	96	384	
VDR Lysis Buffer	30 mL	110 mL	15°C to 25°C
HSW Buffer	22 mL	88 mL	15°C to 25°C
Nuclease-free Water	35 mL	150 mL	15°C to 25°C
Pro K Solution	1.1 mL	4.4 mL	2°C to 8°C
Carrier RNA	1 mg	4 x 1 mg	-20°C
MAG-S1 Particles	1.1 mL	4.4 mL	2°C to 8°C

Pro K Solution comes in a ready-to-use solution. All components are stable for 16 months when stored accordingly. Avoid exposure to direct sunlight or extreme temperatures. When working with these buffers, always wear suitable personal protective equipment such as safety glasses, laboratory coat, and gloves. Be careful to avoid contact with skin and eyes. In the case of such contact, wash immediately with water. If necessary, seek medical assistance. For more information, please consult the appropriate SDS (Safety Data Sheet).

Materials to be Supplied by the User

- 96-well magnetic beads separation device (Corning IMAG-96P) or 1.5 mL/2.0 mL magnetic beads separation device (Corning IMAG-12T).
- 96-well microplate or deep well microplate compatible with the magnetic bead separation device.
  - Recommend using the 1.5 mL microcentrifuge tube (Corning MCT-150-C-S) with the 1.5 mL magnetic device (Corning IMAG-12T) or the deep well plate (Corning P-DW-20) with the 96-well magnetic device (Corning IMAG-96P).
- Isopropanol
- 70% (non-denatured) ethanol

Reagent Preparation

- Vortex MAG-S1 beads to ensure they are in suspension prior to initial use.
- Add Ethanol to the HSW Buffer (28 mL for Corning MAG-VNA-S and 112 mL for Corning MAG-VNA-M) and mix by shaking for a few seconds. To avoid ethanol evaporation, be sure and close the bottle tightly after each use to avoid ethanol evaporation.
- Add 1 mL of nuclease-free water to the tube containing lyophilized carrier RNA to obtain a solution of 1 µg/µL. Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at -20°C. Do not freeze-thaw the aliquots of carrier RNA more than 3 times.

Perform Total Nucleic Acid Purification Using 200 µL Sample

1. Perform all of the following steps at room temperature.
2. Vortex beads vigorously to ensure they are homogenous.
3. Prepare lysis mastermix.

Reagent	Per Sample
VDR Lysis Buffer	240 µL
Carrier RNA (1 µg/µL)	8 µL
Isopropanol	280 µL

4. Transfer 528 µL lysis mastermix to each sample well.
5. Add 200 µL sample to each sample well. Mix by vortexing for 1 minute or pipet mix 15 to 20 times. If sample is frozen, allow to thaw to room temperature.
   NOTE: If sample is less than 200 µL, bring volume up to 200 µL with nuclease-free water.
6. Add 10 µL MAG-S1 particles and 10 µL Pro K solution to each well. Mix by shaking for 5 minutes with a vortex mixer (Corning 6776).
   NOTE: Shake well to resuspend the MAG-S1 particles before use.
7. To magnetize the MAG-S1 particles, place the sample plate on the magnetic separation device for 10 minutes.
8. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.
   NOTE: Do not disturb the attracted beads while aspirating the supernatant.
9. Remove the plate from the magnetic separation device.
10. Add 400 µL HSW buffer to each sample and pipet mix 15 times to resuspend the MAG-S1 particles.
11. Place the sample plate back on the magnetic separation device and wait 5 minutes or until the magnetic beads clear from solution.
12. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.
    NOTE: Do not disturb the attracted beads while aspirating the supernatant.
13. Remove the plate from the magnetic separation device.
14. Add 500 µL 70% ethanol to the sample and pipet mix 15 times to resuspend the MAG-S1 particles.
    NOTE: Complete resuspension of the MAG-S1 particles is crucial for obtaining purity.
15. Place the sample plate back on the magnetic separation device and wait 5 minutes or until the magnetic beads clear from solution.
16. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.
    NOTE: Do not disturb the attracted beads while aspirating the supernatant.

17. Repeat Steps 12 through 15 for a second wash.
18. With the plate still on the magnetic separation device, dry the beads by incubating for 10 minutes at room temperature. **NOTE:** It is critical to completely remove any residual liquid from each well.
19. Remove the plate from the magnetic separation device. To completely resuspend the MAG-S1 magnetic particles, add 50 to 100 µL nuclease-free water to each well and pipet mix 25 times. **NOTE:** Complete resuspension of the MAG-S1 particles is crucial for obtaining purity.
20. Incubate at room temperature for 10 minutes.
21. Place the sample plate back on the magnetic separation device and wait 5 minutes or until the magnetic beads clear from solution.
22. Transfer the eluate (cleared supernatant containing the RNA/DNA) to a new microplate for storage. **NOTE:** The purified nucleic acid is ready for immediate use. Alternatively, store the plate at -20°C for long term storage.

For more specific information on claims, visit the Certificates page at **www.corning.com/lifesciences**.

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