Axygen® AxyPrep™ Nucleic Acid Kit

Protocol

A Corning Brand

Introduction

The Axygen AxyPrep Mag Viral Nucleic Acid (VNA) kit is designed for rapid and reliable isolation of DNA from whole blood, serum, plasma, saliva and other bodily fluids. High-quality DNA is suitable for direct use in most downstream applications such as amplifications and enzymatic reactions. The kit can be adapted for use on most liquid handling workstations.

Process Overview

1. Samples are lysed in a specially formulated buffer containing detergent.
2. Nucleic acid is bound to the surface of **MAG-S1 Particles** under proper conditions.
3. Proteins and cellular debris are efficiently washed.
4. Pure RNA/DNA are eluted in **Nuclease-Free Water** or low ionic strength buffer.
5. Purified RNA/DNA can be directly used in downstream applications without the need for further purification.

Materials Supplied in the Kit

- **MAG-S1 Particles:** Magnetic beads. Store at 2 to 8°C upon arrival for up to 12 months. DO NOT FREEZE.
- **VDR Lysis Buffer:** Lysis buffer. Store at room temperature for up to 12 months.
- **HSW Buffer:** Wash buffer. Store at room temperature for up to 12 months.
- **VDR Wash Buffer:** RNA purification agent. Store at -20°C for up to 12 months.
- **Nuclease-Free Water:** Elution buffer. Store at room temperature for up to 12 months.
- **Pro K Solution:** Digestive enzyme. Store at room temperature for up to 12 months.

Caution

All components are stable for a period of at least 12 months from the date of manufacture when stored accordingly. Avoid exposure to direct sunlight or extreme temperatures. When working with the 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 safety datasheet (SDS).

Hardware and Consumables

	Cat. No.	
	MAG-VNA-S	**MAG-VNA-M**
Kit Size	96 preps	384 preps
MAG-S1 Particles	1.1 mL	4.4 mL
VDR Lysis Buffer	30 mL	110 mL
HSW Buffer	22 mL	88 mL
VDR Wash Buffer	1 mg	4 mg
Nuclease-Free Water	35 mL	150 mL
Pro K Solution	1.1 mL	4.4 mL
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Materials to Be Supplied by the User

- Benchtop microcentrifuge capable of 12,000 xg, such as the Corning® LSE™ High Speed Microcentrifuge (Corning, Cat. No. 6765-HS)
- Magnetic separation device for 96-well plate (Corning, Cat. No. IMAG-96P)
- 96 well deep well plates compatible with the magnetic bead separation block
- 80% and 100% ethanol
- Isopropanol (100%)

Preparation Before Experiment

1. Prepare **VDR Wash Buffer**. Measure 200 µg **VDR Wash Buffer** to a microcentrifuge tube. Add 200 µL **Nuclease-Free Water** to the tube to obtain a solution of 1 µg/µL. Dissolve the **VDR Wash Buffer** thoroughly. Aliquot and store at -20°C.

   ***Note*:** Avoid freeze thawing aliquots of **VDR Wash Buffer** more than 3 times.

2. Prepare a **Lysis Master Mix**. Prepare **VDR Wash Buffer** following instructions in step 1 before proceeding.

   - For VNA - 50 µL protocol: Mix 60 µL **VDR Lysis Buffer** and 2 µL **VDR Wash Buffer**. Add the mixture to 70 µL Isopropanol..
   - For VNA - 200 µL protocol: Mix 240 µL **VDR Lysis Buffer** and 2 µL **VDR Wash Buffer**. Add the mixture to 280 µL Isopropanol.

3. Prepare **HSW Buffer** by adding ethanol according to the chart below. Use 100% ethanol.

Catalog No.	Component	Add 100% Ethanol	Storage
MAG-VNA-S	HSW Buffer	28 mL	15 to 25°C
MAG-VNA-M	HSW Buffer	112 mL	15 to 25°C

Protocol for AxyPrep™ Mag Viral Nucleic Acid Kit – 50 µL

1. Binding Step

   a. Transfer 132 µL **Lysis Master Mix** to each sample well.

   b. Add 50 µL plasma or serum to each sample well and pipet mix 15 times.

   ***Note*:** If sample is frozen, allow to thaw to room temperature.

   ***Note*:** If sample is less than 50 µL, bring volume up to 50 µL with **Nuclease-Free Water**.

   b. Add 5 µL **MAG-S1 Particles** and 5 µL **Pro K Solution** to each well and pipet mix 15 times.

   c. Place the sample processing plate on the magnetic separation device to separate the **MAG-S1 Particles** for 10 minutes.

   d. 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.

2. Washing Step

   a. Remove the plate from the magnetic separation device. Add 200 µL **HSW Buffer** to each sample and pipet mix 15 times to resuspend the **MAG-S1 Particles**.

   b. Place the sample processing plate on the magnetic separation device to separate the **MAG-S1 Particles** for 5 minutes or until the beads clear from the solution.

   c. 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.

   d. Remove the plate from the magnetic separation device. Add 200 µL 80% ethanol to each sample and pipet mix 15 times to resuspend the **MAG-S1 Particles**.

   f. Place the sample processing plate on the magnetic separation device for 5 minutes or until the beads clear from the solution.

   g. 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.

   h. Repeat steps 2d to 2g for a second wash.

   i. Dry the beads by incubating at room temperature for 7 minutes with the plate still on the magnetic separation device.

3. Elution Step

a. Remove the plate from the magnetic separation device. Add 20 to 50 µL of **Nuclease-Free Water** to each well and pipet mix 25 times to completely resuspend the **MAG-S1 Particles**.

b. Incubate at room temperature for 10 minutes.

c. Place the sample processing plate on the magnetic separation device and wait for 5 minutes or until the beads clear from the solution.

d. Transfer the eluate (cleared supernatant containing the DNA) to a new microplate for storage. Store DNA at -20°C.

Protocol for Axygen® AxyPrep™ Mag Viral Nucleic Acid Kit – 200 µL

1. Binding Step

a. Transfer 528 µL **Lysis Master Mix** to each sample well.

b. Add 200 µL plasma or serum to each sample well. Mix by vortexing for 1 minute or pipet mix 15 to 20 times.

***Note*:** 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**.

2. Washing Step

a. Add 10 µL **MAG-S1 Particles** and 10 µL **Pro K Solution** to each well and mix by shaking for 5 minutes.

b. Place the sample processing plate on the magnetic separation device for 10 minutes to separate the **MAG-S1 Particles.**

c. 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.

d. Remove the plate from the magnetic separation device. Add 400 µL **HSW Buffer** to each sample and pipet mix 15 times to resuspend the **MAG-S1 Particles**.

e. Place the sample processing plate back on the magnetic separation device and wait for 5 minutes or until the beads clear from solution.

f. 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.

g. Remove the plate from the magnetic separation device. Add 500 µL 80% ethanol to each sample and pipet mix 15 times to resuspend the **MAG-S1 Particles**.

h. Place the sample processing plate on the magnetic separation device to separate the **MAG-S1 Particles** for 5 minutes or until the beads clear from the solution.

i. 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.

j. Repeat steps 2d to 2i for a second wash.

k. Dry the beads by incubating at room temperature for 10 minutes with the plate still on the magnetic separation device.

3. Elution Step

a. Remove the plate off the magnetic separation device. Add 50 to 100 µL **Nuclease-Free Water** to each sample and pipet mix 25 times to resuspend the **MAG-S1 Particles**.

b. Incubate the sample plate at room temperature for 10 minutes.

c. Place the sample processing plate on the magnetic separation device to separate the **MAG-S1 Particles** for 5 minutes or until the beads clear from the solution.

d. Transfer the eluate (cleared supernatant containing the DNA) to a new plate. Store DNA at -20°C.

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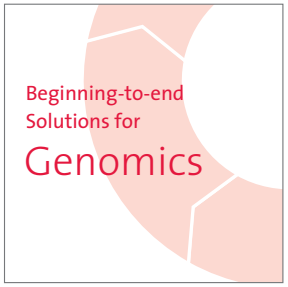

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