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Introduction

The E-Z 96 Mag-Bind™Viral DNA/RNA Kit is designed for rapid and reliable isolation of viral DNA and RNA from serum, plasma, and other cell-free body fluids. The Mag-Bind™ Magnetic Beads technology provide high quality RNA and RNA, which is suitable for direct use in most downstream applications such as amplifications and enzymatic reactions. This system can be easily adapted with an automated system and the procedure can be scaled up or down.

Principle

If using the E-Z 96 Mag-Bind™ Viral DNA/RNA Kit for the first time, please read this booklet to become familiar with the procedure and its various modifications. Samples are lysed in a specially formulated buffer containing detergent and chaotropic salt. After adjust the buffer condition, nucleic acids (DNA/RNA) will form a complex with magnetic beads.. The beads/nucleic acids complex is separated from lysates using a magnet. Proteins and cellular debris are efficiently washed away by a washing step. Purified DNA and RNA are then eluted in nuclease-free water or low ionic strength buffer. DNA and RNA purified with this method can be directly used in most downstream applications without the need for further purification.

Storage and Stability

E-Z 96 Mag-Bind™ Viral DNA/RNA Kit are stable for at least 12 months from date of purchase. During shipment, or storage in cool ambient conditions, precipitates may form in the MVL Buffer. Mag-Bind™ Particle R need to be stored at 4-8° C.

Kit Contents

Product	M6246-00	M7246-01	M6246-02
Purification	1 x 96	4 x 96	12 x 96
Process Plate	1	4	12
Mag-Bind™ Particles R	1 mL	4 mL	12 mL
Buffer MVL*	50 ml	4 x 50 mL	3 x 200 mL
Proteinase K	40 mg	160 mg	3 x 160 mg
Buffer MBW	25 ml	100 ml	300 ml
SPR Wash Buffer	25 mL	125 mL	3 x 125 mL
DEPC-Water	50 mL	200 mL	150 mL
Carrier RNA	1 mg	2 mg	2 mgl
User Manual	1	1	1

* MVL Contains guanidine salts. Do not use bleach as disinfectant.

Before Starting

IMPORTANT	**SPR Wash Buffer** must be diluted with absolute ethanol before use.	
	M6246-00	Add 75 mL 100% ethanol
	M6246-01	Add 375 mL 100% ethanol
	M6246-02	Add 375 mL 100% ethanol
	Buffer MBW must be diluted with isopropanol before use.	
	M6246-00	Add 25 ml Isopropanol
	M6246-01	Add 100 ml Isopropanol
	M6246-02	Add 300 ml Isopropanol
	Dissolve Proteinase K as following and store at -20°C**.**	
	M6246-00	Add 1ml DEPC-Water
	M6246-01	Add 4 ml DEPC-Water
	M6246-02	Add 4 ml DEPC-Water

Please take a few minutes to read this booklet thoroughly and become familiar with the protocol. Prepare all materials required before starting in order to minimize RNA degradation. ***Wear gloves/protective goggles and take great care when working with chemicals*.**

- Whenever working with RNA, always wear latex gloves to minimize RNase contamination. Use only clean RNase-free disposable plastic pipette tips when using the supplied reagents.
- During the procedure work carefully but quickly.
- Under cool ambient conditions, crystals may form in MRB Bufer This is normal and the bottle should be warmed to re-dissolve the salt.
- Prepare Carrier RNA: Dissolve Carrier RNA with DEPC-Water to the final concentration at 1mg/ml. Dissolve the dissolved Carrier RNA into convenient aliquot and store at -20°C. Add 3 µl Carrier RNA for each milliliter of MSL Buffer before use.

Material and Equipments supplied by User

Have the following reagents and supplies ready before starting procedure:

- Magnetic separation device for 96 deep well plate (MSD-01b)
- Nuclease-free 96 deep well plate (1.2 ml)
- Multichannel pipette
- Nuclease-Free pipetting tips
- Absolute ethanol (96-100%)
- Isopropanol

Mag-Bind™ Viral DNA/RNA Isolation Protocol (for 200ul serum or plasma)

The following protocol is designed for isolating viral DNA and RNA from 200 µl cell-free body fluids such as plasma, or serum. The protocol can be easily scale up to process up to 400 µl sample by using appropriate processing plate and suitable magnetic separation device.

1. **Add 200 ul sample (serum, plasma or other cell-free body fluids) into appropriate Processing Plate (not supplied) followed by adding 10 ul Proteinase K solution and 10 ul Mag-Bind™ Particle RV**.

   Note: Proteinase K and **Mag-Bind™** Particles R can be prepared as master mix and

add to each at one time. For 96 samples, mix 1 vial **Mag-Bind™** Particle R (1 ml) with 1 ml Proteinase K solution. Add 20 µl beads/proteinase K mix for each blood sample.

2. **Add 200 µl MVL/Carrier RNA Buffer mix to the sample. Mix throughly by pipetting or vortexing .** Incubate at room temperature for 10 minutes.

   Note: see the instruction for preparing Carrier RNA on page 4.

3. **Add 400ul isopropanol to each sample, mix throughly by pipetting or vortexing. Incubate 3-5minutes at room temperature.**

4. **Place the Processing Plate on a magnetic separation device to magnetize the magnetic particles.** Leave the plate on the magnet until all the magnetic particles are pelleted. The capture time is depend on the sample type and magnetic stand used.

5. **Carefully aspirate and discard the supernatant without disturbing the magnetic particles.**

6. **Remove the Processing Plate containing the magnetic particles from the magnetic separation device.** Add 300 µl of Buffer MBW and resuspend magnetic particles pellet by pipetting up and down 20 times or vortex the plate at maximum speed for 1 minute.

7. **Place the plate onto a magnetic separation device to magnetize the magnetic particles.** Aspirate and discard the cleared supernatant after the lysate is cleared.

8. **Remove the plate containing the magnetic particles from the magnetic separation device.** Add 400 µl of SPR Wash Buffer to each well and resuspend magnetic particles pellet by pipetting up and down or vortexing for 30 seconds**.**

9. **Place the plate onto a magnetic separation device to magnetize the magnetic particles.** Aspirate an discard the cleared supernatant after the lysate is cleared.

10. **Add another 400 µl of SPR Wash Buffer and resuspend magnetic particles pellet by pipetting up and down for 20 times or vortexing at maximum speed for 30 seconds.**

11. **Place the plate onto a magnetic separation device to magnetize the Mag-Bind™ particles.**

12. **Aspirate and discard the cleared supernatant**. Carefully remove any liquid drop from tube. Leave the tube on the magnet stand. Air dry the magnetic particles at room

temperature for 7-10 minutes.

13. **Add 20-50 µl DEPC Treated water and resuspend magnetic particles pellet by vortexing or pipetting up and down for 20 times**. Incubate at room temperature for 3 minutes.

14. **Place the tube onto a magnetic separation device to magnetize the Mag-Bind™ particles**.

15. Transfer the cleared supernatant contains purified DNA and RNA into a new RNase-free microplate .

Mag-Bind™ Viral DNA/ RNA Isolation Protocol (for 400ul serum or plasma

The following protocol is designed for isolating viral DNA and RNA from 400 µl cell-free body fluids such as plasma, or serum. This protocol should use appropriate processing plate and suitable magnetic separation device.

1. **Add 400 ul sample (serum, plasma or other cell-free body fluids) into appropriate Processing Plate (not supplied) followed by adding 10 ul Proteinase K solution and 10 ul Mag-Bind™ Particle RV**.

   Note: Proteinase K and **Mag-Bind™** Particles R can be prepared as master mix and add to each at one time. For 96 samples, mix 1 vial **Mag-Bind™** Particle R (1 ml) with 1 ml Proteinase K solution. Add 20 µl beads/proteinase K mix for each blood sample.

2. **Add 400 µl MVL/Carrier RNA Buffer mix to the sample. Mix throughly by pipetting or vortexing .** Incubate at room temperature for 10 minutes.

   Note: see the instruction for preparing Carrier RNA on page 4.

3. **Add 800ul isopropanol to each sample, mix throughly by pipetting or vortexing. Incubate 3-5minutes at room temperature.**

4. **Place the Processing Plate on a magnetic separation device to magnetize the magnetic particles.** Leave the plate on the magnet until all the magnetic particles are pelleted. The capture time is depend on the sample type and magnetic stand used.

5. **Carefully aspirate and discard the supernatant without disturbing the magnetic particles.**

6. **Remove the Processing Plate containing the magnetic particles from the**

magnetic separation device. Add 400 µl of Buffer MBW and resuspend magnetic particles pellet by pipetting up and down 20 times or vortex the plate at maximum speed for 1 minute.

7. **Place the plate onto a magnetic separation device to magnetize the magnetic particles.** Aspirate and discard the cleared supernatant after the lysate is cleared.

8. **Remove the plate containing the magnetic particles from the magnetic separation device.** Add 500 µl of SPR Wash Buffer to each well and resuspend magnetic particles pellet by pipetting up and down or vortexing for 30 seconds**.**

9. **Place the plate onto a magnetic separation device to magnetize the magnetic particles.** Aspirate an discard the cleared supernatant after the lysate is cleared.

10. **Add another 500 µl of SPR Wash Buffer and resuspend magnetic particles pellet by pipetting up and down for 20 times or vortexing at maximum speed for 30 seconds.**

11. **Place the plate onto a magnetic separation device to magnetize the Mag-Bind™ particles.**

12. **Aspirate and discard the cleared supernatant**. Carefully remove any liquid drop from tube. Leave the tube on the magnet stand. Air dry the magnetic particles at room temperature for 7-10 minutes.

13. **Add 50-100 µl DEPC Treated water and resuspend magnetic particles pellet by vortexing or pipetting up and down for 20 times**. Incubate at room temperature for 3 minutes.

14. **Place the tube onto a magnetic separation device to magnetize the Mag-Bind™ particles**.

15. Transfer the cleared supernatant contains purified DNA and RNA into a new RNase-free microplate .

Trouble Shooting

Problem	Likely Cause	Suggestions
Low RNA yields	Incomplete resuspension of magnetic particles	Resuspend the magnetic particles by vortexing before use.
	RNA degraded during sample storage	Make sure the sample is properly stored and make sure the samples are processed immediately after collection or removal from storage.
	SPR Buffer were not prepared correctly.	Prepare the SPR Buffer by adding ethanol according to instruction
	Loss of magnetic beads during operation	Be careful not to remove the magnetic beads during the operation
	Undissolved particles in the cell lysate cause congregation of magnetic beads	make sure the lysate is clear of particles before adding magnetic beads. Do not over dry the magnetic beads
No RNA eluted.	SPR and MBW Wash Buffer Concentrate not diluted with absolute ethanol and isopropanol.	Prepare MBW and SPR Wash Buffer Concentrate as instructed on the label.
Problem with downstream application	Insufficient RNA was used	1. RNA in the sample is already degraded; do not freeze and thaw the sample more than once or store at room temperature for too long 2. Quantify the purified DNA/RNA accurately and use sufficient elute.
Carryover of the magnetic beads in the elution	Carryover from the magnetic beads in the eluted RNA will not effect downstream applications.	To remove the carryover magnetic beads from eluted DNA/RNA, simply magnetize the magnetic beads and carefully transfer to a new tube or plate.