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HighPrep Total RNA Plus Kit - DX

Isolation of RNA from a Variety of Sample Types

Catalog Nos. HPTOR-R5E, HPTOR-R50E, HPTOR-R100E, HPTOR-R100x4E
Manual Revision 0
WI-72-65


- RNA isolation from mammalian tissues, cultured cells, buccal cells, and whole blood
- Magnetic bead-based chemistry

Instructions For Use

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For *in vitro* diagnostic procedures.

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Product Description

The HighPrep Total RNA Plus Kit - DX is specially designed for purifying total RNA from a wide variety of sample types including mammalian tissues, cultured cells, buccal cells, and whole blood. The kit isolates high-quality total RNA from 5-30 mg of tissue, 200 µL of whole blood, 1 x 10⁶ of cultured cells, and 200 µL of swab lysate. Purified RNA is suitable for all major downstream applications such as RNA-Seq, RT-PCR, NGS, and hybridization applications. This protocol can be used for manual procedure as well as a guideline for adapting the kit to automated liquid handling instruments.

This product is intended to be used by qualified and trained laboratory professionals only.

Process

The HighPrep Total RNA Plus Kit - DX uses a simple 4 step procedure: Lyse+Bind-Wash-Elute. Tissue, blood, or cells are lysed, and released RNA binds to the MAG-R4 Particles in one step. Utilizing a magnetic separation device, the bound RNA is separated from the solution and washed. Genomic DNA is removed with a DNase Digestion step. After 2 wash steps, RNA is eluted from the magnetic beads.

Kit Contents and Storage

HighPrep Total RNA Plus Kit - DX Catalog No.*	HPTOR-R5E	HPTOR-R50E	HPTOR-R100E	HPTOR-R100x4E	Storage
Number of Preps	5	50	100	400	
Solution A	5 mL	50 mL	100 mL	400 mL	15-25°C
LB Buffer	2.8 mL	28 mL	56 mL	224 mL	15-25°C
CE Buffer ¹	800 µL	8 mL	16 mL	64 mL	15-25°C
RW1 Buffer ¹	2 mL	20 mL	40 mL	160 mL	15-25°C
RB2 Buffer ¹	2 mL	20 mL	40 mL	160 mL (80 mL x 2)	15-25°C
Pro K Solution ²	110 µL	1.1 mL	2.2 mL	8.8 mL	2-8°C
DNase I ³	11 µL	110 µL	220 µL	880 µL	-20°C
DNase I Digestion Buffer	600 µL	6 mL	12 mL	48 mL	15-25°C
RNA Elution Buffer	1 mL	10 mL	16 mL	64 mL	15-25°C
MAG-R4 Particles ⁴	55 µL	550 µL	1.1 mL	4.4 mL	2-8°C

¹Ethanol must be added prior to use. See preparation of reagents section.

*Once opened, reagents are usable until the expiration date on the product label. Be sure to close the lid firmly before storing reagents for later use.

Shipping and Storage

- ²Pro K Solution comes in a ready to use solution. Ships at room temperature. Store at 2-8°C.
- ³DNase I ships at room temperature. Store at -20°C.
- ⁴MAG-R4 Particles ship at room temperature. Store at 2-8°C.

Safety Information

Any consumables, including plates, tubes, etc., used to process samples with infectious or microbial hazards should be disposed of in an appropriate biohazard waste bin. When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate safety data sheets (SDSs). The SDS can be downloaded from the "Product Documents" tab when viewing this product at www.magbiogenomics.com.

Preparation of Reagents

Prepare the following components for each kit before use.

Catalog No.	Component	Add 100% Ethanol*	Storage
HPTOR-R5E	CE Buffer	2 mL	Room Temp 15-25°C
	RW1 Buffer	1.25 mL	Room Temp 15-25°C
	RB2 Buffer	8 mL	Room Temp 15-25°C

Catalog No.	Component	Add 100% Ethanol*	Storage
HPTOR-R50E	CE Buffer	20 mL	Room Temp 15-25°C
	RW1 Buffer	12.5 mL	Room Temp 15-25°C
	RB2 Buffer	80 mL	Room Temp 15-25°C

Catalog No.	Component	Add 100% Ethanol*	Storage
HPTOR-R100E	CE Buffer	40 mL	Room Temp 15-25°C
	RW1 Buffer	25 mL	Room Temp 15-25°C
	RB2 Buffer	160 mL	Room Temp 15-25°C

Catalog No.	Component	Add 100% Ethanol*	Storage
HPTOR-R100x4E	CE Buffer	160 mL	Room Temp 15-25°C
	RW1 Buffer	100 mL	Room Temp 15-25°C
	RB2 Buffer	320 mL per bottle	Room Temp 15-25°C

*Ensure bottle/tube lid is closed tightly when preparing and storing reagents.

Amounts of Starting Material

Use the amounts of starting material indicated in the following table.

Sample Type	Amount
Tissues	30 mg
Cultured Cells	1x10 ⁶ pellet
Blood	200 µL
Buccal cells	4 buccal swabs

Protocol: Tissues & Cultured Cells (1.5/2.0 mL tube format)

Equipment and Reagents to Be Supplied by the User

- Nuclease-free 1.5/2.0 mL microcentrifuge tubes
- Magnetic separation device for 1.5/2.0 mL microcentrifuge tubes (see page 11)
- Equipment for disrupting and homogenizing tissue
- Water bath, incubator, or heat block capable of 65°C
- Ethanol (100%)

Things to do Before Starting

- Ensure that the work area is RNase free
- Prepare all reagents according to the instructions on page 2
- Preset water bath, incubator, or heating block to 65°C

Protocol


 Bring the **MAG-R4 Particles** to room temperature for at least 30 minutes before use.

1. Homogenize the tissue or cells samples using an appropriate method. See below examples of common homogenization methods:

- For tissue samples

1) Mortar and pestle: Collect ~30 mg of fresh or preserved tissue sample in a mortar and freeze in liquid nitrogen. Grind the tissue using a clean pestle. Transfer the ground powder and liquid nitrogen into a 1.5 mL tube and allow the liquid nitrogen to evaporate. Add 500 μ L of **LB Buffer** and vortex immediately and thoroughly.


2) Bead-beating: Grind the ~30 mg of tissue in 500 μ L of **LB Buffer** and use a commercial bead-beater following the manufacturer's instructions. Parameters such as grinding speed, duration, temperature, and type of beads, etc. may need to be optimized. Refer to the manufacturer's manual for additional instructions.

 **Pro K Solution** is not necessary when using a clean mortar or bead-beating to grind tissue samples



- For cultured cells

Resuspend the 1×10^6 cultured cell pellet in 500 μ L of **LB Buffer** by vortexing or pipetting up-and-down thoroughly. Add 20 μ L of **Pro K Solution**, mix immediately by vortexing for 20 seconds, and incubate the sample at room temperature for 5 minutes. Vortex briefly once during incubation. If desired, using a rotor-stator homogenizer or passing through a 20-gauge needle may increase the yield.

2. Centrifuge the sample at 10,000 x g for 10 minutes at 4°C. Transfer 400 μ L of the clear lysate to a new tube. Do not disturb the debris pellet.

 If the clear lysate is less than 400 μ L, bring the volume up to 400 μ L with **LB Buffer** or add the same volume of diluted **CE Buffer** at step 3.

3. Add 400 μL of **CE Buffer** and 10 μL of **MAG-R4 Particles** to each sample, vortex to mix thoroughly, and incubate at room temperature for 10 minutes.
 ⚠ **CE Buffer** must be diluted with Ethanol prior to use. Complete resuspension of the **MAG-R4 Particles** is critical for obtaining high quality RNA.
4. Place the sample tube on a magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes or until the magnetic particles are completely cleared from the solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.
5. Add 600 μL of **RW1 Buffer** to the sample and resuspend the **MAG-R4 Particles** by vortexing at maximum speed for 1 minute or by pipetting up and down 10 times.
 ⚠ **RW1 Buffer** must be diluted with Ethanol prior to use. Complete resuspension of the **MAG-R4 Particles** is critical for obtaining high quality RNA.
6. Place the sample tube on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes or until the magnetic particles are completely cleared from the solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.
7. Add 600 μL of **RB2 Buffer** to the sample and resuspend the **MAG-R4 Particles** by vortexing at maximum speed for 1 minute or by pipetting up and down 10 times.
 ⚠ **RB2 Buffer** must be diluted with Ethanol prior to use. Complete resuspension of the **MAG-R4 Particles** is critical for obtaining high quality RNA.
8. Place the sample tube on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes or until the magnetic particles are completely cleared from the solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.
 ⚠ All liquid must be aspirated at this step. It is helpful to remove all liquid from the well then wait for one minute and remove any residual liquid from the well using a fine pipette tip.
9. Leave the tube on the magnetic separation device for 5 minutes to air dry the **MAG-R4 Particles**.
10. While the sample is drying, prepare the **DNase I** mixture. For each sample, gently mix 98 μL of **DNase I Digestion Buffer** and 2 μL of **DNase I**.
11. With the tube off the magnetic separation device, add 100 μL of the **DNase I** mixture to each sample. Gently mix by pipetting up and down to fully resuspend the **MAG-R4 Particles**. Incubate the samples at room temperature for 10 minutes.
 ⚠ Avoid extensive vortexing or pipetting as this may denature the **DNase I**.
12. With the tube off the magnetic separation device, add 600 μL of **RB2 Buffer** to the sample and resuspend the **MAG-R4 Particles** by vortexing at maximum speed for 1 minute or by pipetting up and down 10 times. Incubate the samples at room temperature for 1 minute.
 ⚠ **RB2 Buffer** must be diluted with Ethanol prior to use. Complete resuspension of the **MAG-R4 Particles** is critical for obtaining high quality RNA.
13. Place the sample tube on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes or until the magnetic particles are completely cleared from the solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.
14. Repeat steps 12-13 for a second RNA wash.
15. Leave the tube on the magnetic separation device for 5-10 minutes to air dry the **MAG-R4 Particles**. Remove any residual liquid with a fine pipette tip.
 ⚠ It is critical to completely remove all liquid from each tube.

16. Add 50-100 μ L of **RNA Elution Buffer**. Completely resuspend the **MAG-R4 Particles** by vortexing at maximum speed for 1 minute or by pipetting up and down 10 times.
 *For concentrated RNA use a low elution volume.*
17. Incubate for 10 minutes at room temperature.
 *Incubation at 65°C may improve the yield for some tissue samples, but it may decrease the sample's RNA integrity number (RIN) values.*
18. Place the tube back on the magnetic separation device and wait 2-5 minutes or until the magnetic particles are completely cleared from the **RNA Elution Buffer**.
19. Transfer the cleared supernatant containing the purified RNA to a new 1.5 mL tube.
20. Store the RNA at -80°C .

Protocol: Whole Blood (1.5/2.0 mL tube format)

Equipment and Reagents to Be Supplied by the User

- Nuclease-free 1.5/2.0 mL microcentrifuge tubes
- Magnetic separation device for 1.5/2.0 mL microcentrifuge tubes (see page 11)
- Water bath, incubator, or heat block capable of 65°C
- Ethanol (100%)


Things to do Before Starting

- Ensure that the work area is RNase free
- Prepare all reagents according to the instructions on page 2
- Preset water bath, incubator, or heating block to 65°C


Protocol

 Bring the **MAG-R4 Particles** to room temperature for at least 30 minutes before use.

1. Add 200 µL of whole blood to a 1.5 mL microcentrifuge tube and 600 µL of **Solution A**.
2. Mix well and centrifuge at 10,000 x g for 5 minutes at 4°C. Decant the supernatant and keep the pellet.
3. Add 200 µL of **Solution A** and mix gently to break the pellet.
4. Centrifuge the sample at 10,000 x g for 5 minutes at 4°C for the final wash. Decant the supernatant and keep the pellet.
5. Add 500 µL of **LB Buffer** to the pellet and 20 µL of **Pro K Solution**. incubate the sample at room temperature for 20 minutes. Vortex briefly once during incubation.
6. Centrifuge the sample at 10,000 x g for 10 minutes at 4°C. Transfer 400 µL of the clear lysate to a new tube. Do not disturb the debris pellet.


 *If the clear lysate is less than 400 µL, bring the volume up to 400 µL with **LB Buffer** or add the same volume of diluted **CE Buffer** at step 7.*

7. Add 400 µL of **CE Buffer** and 10 µL of **MAG-R4 Particles** to each sample. Vortex to mix thoroughly and incubate at room temperature for 10 minutes.

 ***CE Buffer** must be diluted with Ethanol prior to use. Complete resuspension of the **MAG-R4 Particles** is critical for obtaining high quality RNA.*

8. Place the sample tube on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes or until the magnetic particles are completely cleared from the solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.

9. Add 600 µL of **RW1 Buffer** to the sample and resuspend the **MAG-R4 Particles** by vortexing at maximum speed for 1 minute or by pipetting up and down 10 times.

 ***RW1 Buffer** must be diluted with Ethanol prior to use. Complete resuspension of the **MAG-R4 Particles** is critical for obtaining high quality RNA.*

10. Place the sample tube on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes, or until the magnetic particles are completely cleared from the solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.
11. Add 600 μL of **RB2 Buffer** to the sample and resuspend the magnetic particles by vortexing at maximum speed for 1 minute or by pipetting up and down 10 times.

⚠ *RB2 Buffer must be diluted with Ethanol prior to use. Complete resuspension of the **MAG-R4 Particles** is critical for obtaining high quality RNA.*
12. Place the sample tube on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes or until the magnetic particles are completely cleared from the solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.

⚠ *All liquid must be aspirated at this step. It is helpful to remove all liquid from the well then wait for one minute and remove any residual liquid from the well using a fine pipette tip.*
13. Leave the tube on the magnetic separation device for 5 minutes to air dry the **MAG-R4 Particles**.
14. While the sample is drying, prepare the **DNase I** mixture. For each sample, gently mix 98 μL of **DNase I Digestion Buffer** and 2 μL of **DNase I**.

⚠ *Avoid extensive vortexing or pipetting as this may denature the **DNase I**.*
15. With the tube off the magnetic separation device, add 100 μL of the **DNase I** mixture to each sample. Gently mix by pipetting up and down to fully resuspend the magnetic particles. Incubate the sample at room temperature for 10 minutes.
16. With the tube off the magnetic separation device, add 600 μL of **RB2 Buffer** to the sample and resuspend the magnetic particles by vortexing at maximum speed for 1 minute or by pipetting up and down 10 times. Incubate the samples at room temperature for 1 minute.

⚠ *RB2 Buffer must be diluted with Ethanol prior to use. Complete resuspension of the **MAG-R4 Particles** is critical for obtaining high quality RNA.*
17. Place the sample tube on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes or until the magnetic particles are completely cleared from the solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.
18. Repeat steps 16-17 for a second RNA wash.
19. Leave the tube on the magnetic separation device for 5-10 minutes to air dry the **MAG-R4 Particles**. Remove any residual liquid with a fine pipette tip.

⚠ *It is critical to completely remove all liquid from each tube.*
20. Add 50-100 μL of **RNA Elution Buffer**. Completely resuspend the **MAG-R4 Particles** by vortexing at maximum speed for 1 minute or by pipetting up and down 10 times.

⚠ *For concentrated RNA use a low elution volume.*
21. Incubate for 10 minutes at room temperature.

⚠ *Incubation at 65°C may improve the yield.*
22. Place the tube back on the magnetic separation device and wait 2-5 minutes or until the magnetic particles are completely cleared from the **RNA Elution Buffer**.
23. Transfer the cleared supernatant containing the purified RNA to a new 1.5 mL tube.
24. Store the RNA at -80°C .

Protocol: Buccal Cells (1.5/2.0 mL tube format)

Equipment and Reagents to Be Supplied by the User

- Nuclease-free 1.5/2.0 mL microcentrifuge tubes
- Magnetic separation device for 1.5/2.0 mL microcentrifuge tubes (see page 11)
- Water bath, incubator, or heat block capable of 65°C
- Ethanol (100%)


Things to do Before Starting

- Ensure that the work area is RNase free
- Prepare all reagents according to the instructions on page 2
- Preset water bath, incubator, or heating block to 65°C


Protocol

 Bring the **MAG-R4 Particles** to room temperature for at least 30 minutes before use.

1. Collect buccal cells in swab medium (follow the manufacturer's instructions for buccal cell collection in swab medium).
2. Centrifuge the cells at 10,000 x g for 10 minutes and remove the supernatant. Retain the pellet.
3. Add 500 µL of **LB Buffer** to the pellet. Mix by pipetting up and down thoroughly 10 times. Incubate the sample at room temperature for 5 minutes. Mix briefly once during incubation.
4. Centrifuge the cells at 10,000 x g for 10 minutes. Transfer the clear lysate to a new tube. Do not disturb the debris pellet.
5. Add **CE Buffer** to the lysate in a 1:1 ratio (i.e. for 500 µL of lysate add 500 µL of **CE Buffer**), and 10 µL of **MAG-R4 Particles** to each sample. Vortex to mix thoroughly and incubate at room temperature for 10 minutes.

 **CE Buffer** must be diluted with Ethanol prior to use. Complete resuspension of the **MAG-R4 Particles** is critical for obtaining high quality RNA.

6. Place the sample tube on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes or until the magnetic particles are completely cleared from the solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.
7. Add 600 µL of **RW1 Buffer** to the sample and resuspend the magnetic particles by vortexing at maximum speed for 1 minute or by pipetting up and down 10 times.

 **RW1 Buffer** must be diluted with Ethanol prior to use. Complete resuspension of the **MAG-R4 Particles** is critical for obtaining high quality RNA.

8. Place the sample tube on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes or until the magnetic particles are completely cleared from the solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.

9. Add 600 μL of **RB2 buffer** to the sample and resuspend the magnetic particles by vortexing at maximum speed for 1 minute or by pipetting up and down 10 times.
⚠ ***RB2 Buffer** must be diluted with Ethanol prior to use. Complete resuspension of the **MAG-R4 Particles** is critical for obtaining high quality RNA.*
10. Place the sample tube on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes or until the magnetic particles are completely cleared from the solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.
⚠ *All liquid must be aspirated at this step. It is helpful to remove all liquid from the well then wait for one minute and remove any residual liquid from the well using a fine pipette tip.*
11. Leave the tube on the magnetic separation device for 5 minutes to air dry the **MAG-R4 Particles**.
12. While the samples are drying, prepare the **DNase I** mixture. For each sample, gently mix 98 μL of **DNase I Digestion Buffer** and 2 μL of **DNase I**.
13. With the tube off the magnetic separation device, add 100 μL of the **DNase I** mixture to each sample. Mix by pipetting up and down to fully resuspend the magnetic beads. Incubate the samples at room temperature for 10 minutes.
⚠ *Avoid extensive vortexing or pipetting as this may denature the **DNase I**.*
14. With the tube off the magnetic separation device, add 600 μL of **RB2 Buffer** to the sample and resuspend the magnetic particles by vortexing at maximum speed for 1 minute or by pipetting up and down 10 times. Incubate the sample at room temperature for 1 minute.
⚠ ***RB2 Buffer** must be diluted with Ethanol prior to use. Complete resuspension of the **MAG-R4 Particles** is critical for obtaining high quality RNA.*
15. Place the sample tube on the magnetic separation device to magnetize the **MAG-R4 Particles** for 2-5 minutes, or until the magnetic particles are completely cleared from the solution. Remove and discard the cleared supernatant. Do not disturb the magnetic particles.
16. Repeat steps 14-15 for a second RNA wash.
17. Leave the tube on the magnetic separation device for 5-10 minutes to air dry the **MAG-R4 Particles**. Remove any residual liquid with a fine pipette tip.
⚠ *It is critical to completely remove all liquid from each tube.*
18. Add 30-50 μL of **RNA Elution Buffer**. Completely resuspend the **MAG-R4 Particles** by vortexing at maximum speed for 1 minute or by pipetting up and down 10 times.
⚠ *For concentrated RNA use a low elution volume.*
19. Incubate for 10 minutes at room temperature.
⚠ *Incubation at 65°C may improve the yield.*
20. Place the tube back on the magnetic separation device and wait 2-5 minutes or until the magnetic particles are completely cleared from the **RNA Elution Buffer**.
21. Transfer the cleared supernatant containing the purified RNA to a new 1.5 mL tube.
22. Store the purified RNA at -80°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via:

Phone: US/Canada, +1 301-302-0144. Europe, +49 7250 33 13 403

Email: US/Canada, support@magbiogenomics.com. Europe, info.europe@magbiogenomics.com

Symptoms	Possible Causes	Comments
Low RNA yield	Incomplete resuspension of the MAG-R4 Particles	Resuspend the MAG-R4 Particles by vortexing before use
	RNA degraded during storage	Make sure the sample is properly stored and make sure the samples are processed immediately after collection or removal from storage
	Low levels of leukocytes	Low white blood cell count will result in reduced yield
	Loss of MAG-R4 Particles during procedure	Be careful not to remove the MAG-R4 Particles during the procedure
	100% Ethanol was not added to the Wash Buffers	Add Ethanol to the Wash Buffers as instructed on page 2
	MAG-R4 Particles not resuspended during binding	Vortex vigorously for 2 minutes after addition of Ethanol and MAG-R4 Particles
Problems with downstream applications	Insufficient RNA was used	RNA in the sample already degraded. Do not freeze/thaw the sample more than once. Do not store at room temperature
	Ethanol carry-over	Dry the MAG-R4 Particles completely before elution
Carryover of the magnetic particles in the eluate	Carryover of the MAG-R4 Particles in the eluted RNA will affect downstream applications	To remove the carryover MAG-R4 Particles from the eluted RNA, simply place the plate on the magnetic separation device and wait until the eluate has cleared. Carefully transfer the RNA eluate to a new 96-well plate

Ordering Information

HighPrep Total RNA Plus Kit - DX

Catalog No.	Product	Description	Preps
HPTOR-R50E	HighPrep Total RNA Plus Kit - DX (50 Preps)	Magnetic bead-based kit for RNA isolation from tissues, cultured cells, buccal cells, and whole blood.	50
HPTOR-R100E	HighPrep Total RNA Plus Kit - DX (100 Preps)		100
HPTOR-R100x4E	HighPrep Total RNA Plus Kit - DX (400 Preps)		400

Related Products

HighPrep RNA Elite - DX

Catalog No.	Product
RC-90005E	HighPrep RNA Elite - DX (5 mL)
RC-90050E	HighPrep RNA Elite - DX (50 mL)
RC-90250E	HighPrep RNA Elite - DX (250 mL)
RC-90500E	HighPrep RNA Elite - DX (500 mL)

Magnetic Separation Devices

Catalog No.	Product
MYMAG-96	Handheld Magnetic Separation Device (96 well microplate format)
MYMAG-96X	Magnetic Separation Device (96 well ring format)
MBMS-12	MagStrip Magnet Stand (1.5 mL x 12)
MBMS-31550	15 mL and 50 mL Magnetic Stand Combo (3 x 15 mL and 3 x 50 mL)



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