TECHNICAL MANUAL

Maxwell® 16 LEV simplyRNA Blood Kit

Instructions for Use of Product
AS1310

Note: To run the simplyRNA blood protocol, you must have Maxwell® 16 firmware version ≥4.95 (Cat.# AS2000) or ≥1.50 (Cat.# AS3000) installed on your instrument, and you must use the Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070). Using the original LEV magnetic rod will result in low yields.

Revised 3/14
TM372

Maxwell® 16 LEV simplyRNA Blood Kit

All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The Maxwell® 16 LEV simplyRNA Blood Kit[a] (Cat.# AS1310) is designed for isolation of total RNA from fresh (not frozen) whole blood collected in EDTA tubes. The kit is used with the Maxwell® 16 Instrument (Cat.# AS2000 or AS3000) configured with the Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070). The simplyRNA blood procedure purifies total RNA with minimal sample handling before automated purification on the Maxwell® 16 Instrument. The low elution volume is used to generate concentrated high-quality RNA suitable for use in downstream applications such as quantitative RT-PCR. The kit provides the reagents required for sample processing and uses prefilled cartridges for purification, maximizing simplicity and convenience. The Maxwell® 16 Instrument can process from 1 to 16 samples in about an hour.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® 16 LEV simplyRNA Blood Kit	**48 preps**	**AS1310**

For Laboratory Use. Sufficient for 48 automated isolations from fresh blood in EDTA collection tubes. Includes:

- 48 Maxwell® 16 LEV Cartridges (MCF)
- 30ml Homogenization Solution
- 20ml Lysis Buffer
- 2 vials DNase I (lyophilized)
- 900µl 1-Thioglycerol
- 50µl Blue Dye
- 25ml Nuclease-Free Water
- 50 LEV Plungers
- 50 Elution Tubes, 0.5ml
- 4 × 100ml Cell Lysis Solution
- 2 × 1ml Proteinase K

Storage Conditions: Upon receipt, remove the 1-Thioglycerol and store at 2–10°C. Store the remaining kit components at room temperature (15–30°C). 1-Thioglycerol also can be stored at room temperature (15–30°C), where it is stable for up to 9 months.

Safety Information: The reagent cartridges contain ethanol, which is flammable. 1-Thioglycerol is toxic. Guanidine thiocyanate and guanidine hydrochloride (components of the Homogenization Solution and Lysis Buffer) are harmful and irritants. The Lysis Buffer also has a possible risk of harm to an unborn child. Wear gloves and follow standard safety procedures while working with these substances.

The Maxwell® 16 reagent cartridges are designed to be used with potentially infectious substances. Wear appropriate protection (e.g., gloves and goggles) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances used with this system.

Note: Bleach reacts with guanidine thiocyanate and should not be added to any sample waste containing the Homogenization Solution.

Available Separately

PRODUCT	SIZE	CAT.#
Cell Lysis Solution	**1L**	**A7933**

3. Before You Begin

3.A. Maxwell® 16 Instrument Hardware and Firmware Setup

To use the Maxwell® 16 LEV simplyRNA Blood Kit, the Maxwell® 16 Instrument must be configured with LEV hardware. If your Maxwell® 16 Instrument contains standard elution volume (SEV) hardware or the original LEV Magnetic Rod, **it must be reconfigured using the Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070;** Figure 1). Using the original LEV magnetic rod will result in low yields. Reconfiguring the instrument is simple. Refer to the *Maxwell® 16 Instrument Operating Manual* specific for your instrument for directions.

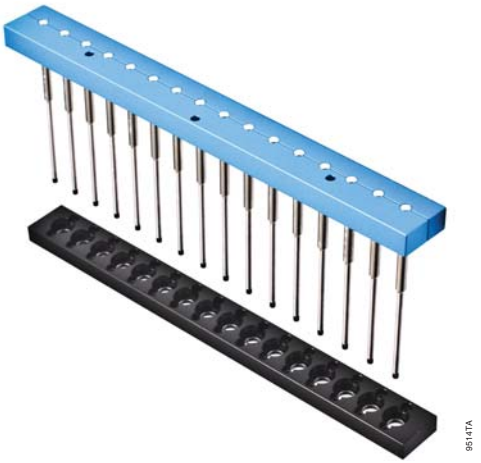

Figure 1. Maxwell 16® High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070).

Failure to change the Maxwell® 16 Instrument hardware to the LEV configuration could result in instrument damage. Using the standard elution volume (SEV) hardware configuration with LEV-configured reagent products will cause damage to the instrument.

Important: To run the simplyRNA blood protocol, you must have Maxwell® 16 firmware version ≥4.95 (Cat.# AS2000) or ≥1.50 (Cat.# AS3000) installed on your instrument.

Capacity: The Maxwell® 16 simplyRNA Blood Kit can process 2.5ml of fresh whole blood per RNA isolation.

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3.B. Preparation of Solutions

Homogenization Solution

To prepare a working solution, add 20µl of 1-Thioglycerol per milliliter of Homogenization Solution. 1-Thioglycerol is viscous, so careful pipetting is required for accurate measurement. Alternatively, add 600µl of 1-Thioglycerol to the 30ml bottle of Homogenization Solution. A volume of 200µl of 1-Thioglycerol/Homogenization Solution is needed for each sample. Before use, chill the 1-Thioglycerol/Homogenization Solution on ice or at 2–10°C.

Note: Store the 1-Thioglycerol/Homogenization Solution at 2–10°C, where it is stable for up to 30 days.

DNase I

Add 275µl of Nuclease-Free Water to the vial of lyophilized DNase I. Invert to rinse DNase off the underside of the cap and swirl gently to mix; do not vortex. Add 5µl of Blue Dye to the reconstituted DNase I as a visual aid for pipetting. Dispense the DNase I solution into single-use aliquots in nuclease-free tubes. Each purification requires 10µl of DNase I solution. Store reconstituted DNase I at –20°C. Do not freeze-thaw reconstituted DNase I more than three times.

3.C. Cartridge Preparation

Cartridges should be prepared shortly before adding the lysate at Step 8 in Section 4.B.

To maintain an RNase-free environment during processing, change gloves before handling cartridges, LEV Plungers and Elution Tubes. Place the cartridges to be used in the Maxwell® 16 LEV Cartridge Rack (Cat.# AS1251). Place each cartridge in the rack with the label side facing away from the Elution Tubes. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument.

Note: If you are processing fewer than 16 samples, center the cartridges on the platform.

1. Place an LEV Plunger in well #8 of each cartridge. Well #8 is the well closest to the Elution Tube.
2. Place 0.5ml Elution Tubes in the front of the Maxwell® 16 LEV Cartridge Rack. Add 50µl of Nuclease-Free Water to the bottom of each Elution Tube. For a more concentrated eluate, as little as 30µl of nuclease-free water may be added to the elution tube, but the total amount of RNA recovered may be reduced.

Notes:

1. If Nuclease-Free Water is on the side of the tube, the elution may be suboptimal.
2. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may not work with the Maxwell® 16 Instrument.

Figure 2. The Elution Tubes are placed in the front of the Maxwell® 16 LEV Cartridge Rack, and 50µl of Nuclease-Free Water is dispensed into each tube.

Figure 3. The LEV plunger is placed in well #8 of the cartridge (the well closest to the Elution Tube), and lysates are placed into well #1 of the cartridge.

4. Purification of Total RNA from Fresh Whole Blood in EDTA Collection Tubes

Materials to Be Supplied By the User

- fresh (not frozen) whole blood in EDTA collection tubes
- vortex mixer
- 15ml tubes (sterile)
- centrifuge with swinging-bucket rotor
- RNase-free, sterile, aerosol-resistant pipette tips

Note: The simplyRNA Blood Kit contains two reagents with the word lysis in their name: **Cell Lysis Solution** (Part# A793A, 100ml) and **Lysis Buffer** (Part# MC501C, 20ml). Please check that you use the correct reagent at each step.

1. Transfer 2.5ml of well mixed fresh (not frozen) whole blood from the EDTA collection tube into a sterile 15ml tube.
2. Add 7.5ml of Cell Lysis Solution (Part# A793A), and invert the tube 5-6 times to mix. This is a differential lysis step; the red blood cells are lysed, leaving the white blood cells intact.
3. Incubate lysates for 10 minutes at room temperature. Twice during the incubation, invert to mix.
4. Centrifuge tube at 3,000 × *g* for 10 minutes.
5. Remove and discard as much of the supernatant as possible without disturbing the visible white pellet. Briefly spin to collect residual liquid at the bottom of the tube, and remove and discard the supernatant with a pipette.
6. Add 200µl of chilled 1-Thioglycerol/Homogenization Solution to the pellet. Mix well with a pipette and/or vortex to ensure complete resuspension of the pellet.
7. Add 200µl of Lysis Buffer (Part# MC501C) and 25µl of Proteinase K to the resuspended pellet. Mix by vortex for 20 seconds.
8. Incubate at room temperature for 10 minutes. During this time prepare cartridges as described in Section 3.C.
9. Add 10µl of DNase I solution (blue solution; prepared as described in Section 3.B) to well #4 of the simplyRNA Blood Cartridge (well #4 contains yellow reagent).
10. Add lysate to well #1 of the simplyRNA Blood cartridge (the well closest to the label on the cartridge).

5. Instrument Run: AS2000 and AS3000 Instruments

5.A. Setup for AS2000 Maxwell® 16 Instruments

Refer to the *Maxwell® 16 Instrument Operating Manual* #TM295 for more detailed information.

Important: To run the simplyRNA blood protocol, you must have Maxwell® 16 firmware version **4.95** or higher installed on your instrument, and you must use the Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070). Using the original LEV magnetic rod will result in low yields.

1. Turn on the Maxwell® 16 Instrument. The instrument will power up, display the firmware version number, proceed through a self-check and home all moving parts.

   Note: Ensure that you are running firmware version 4.95 or higher.

2. Verify that the instrument settings indicate an "LEV" hardware configuration and "Rsch" operational mode setting.

   Note: Failure to change the Maxwell® 16 Instrument hardware to the LEV configuration could result in instrument damage.

3. Select "Run" on the Menu screen, and press the Run/Stop button to select the method.

4. Select "RNA", select "simplyRNA", then select "simplyRNA Blood" on the Menu screen. Next select "OK" at the Verification screen.

5. Open the door when prompted. Press the Run/Stop button to extend the platform.

Warning: Pinch point hazard.

6. Transfer the Maxwell® 16 LEV Cartridge Rack containing prepared cartridges on the Maxwell® 16 Instrument platform. Ensure that the rack is placed in the Maxwell® 16 Instrument with the Elution Tubes closest to the door. The rack will only fit in the instrument in this orientation. If you have difficulty fitting the rack on the instrument platform, check that the rack is in the correct orientation. Ensure that the cartridge rack is level on the instrument platform.

   Note: Hold the Maxwell® 16 LEV Cartridge Rack by the sides to avoid dislodging cartridges from the rack.

7. Verify that samples were added to well #1 of the cartridges, cartridges in the rack are loaded on the instrument, Elution Tubes are present with 50µl of Nuclease-Free Water and LEV Plungers are in well #8. Well #4 should be green to indicate that DNase was added.

Note: Failure to add DNase will result in DNA in the eluate.

8. Press the Run/Stop button. The platform will retract. Close the door.

Warning: Pinch point hazard.

5.A. Setup for AS2000 Maxwell® 16 Instruments (continued)

9. The Maxwell® 16 Instrument will immediately begin the purification run. The screen will display the steps performed and the approximate time remaining in the run.

   Notes:

   1. Pressing the Run/Stop button or opening the door will pause the run.
   2. If the run is abandoned before completion, the instrument will wash the particles off the plungers and eject the plungers into well #8 of the cartridge. To continue processing the samples, rinse any particles off the plunger into the last well used. Discard the used plungers. Put new plungers into well #8, and start the run from the beginning.

10. When the automated purification run is complete, the LCD screen will display a message that the method has ended.

End of Run

11. Follow the on-screen instructions at the end of the method to open door. Verify that the plungers are located in well #8 of the cartridge at the end of the run. If the plungers are not removed from the magnetic plunger bar, push them down by hand to remove them.
12. Press the Run/Stop button to extend the platform out of the instrument.
13. Remove the Maxwell® 16 LEV Cartridge Rack from the instrument. Remove Elution Tubes containing total RNA, and close the tubes.
14. If paramagnetic particles are present in the elution tubes, centrifuge at 10,000 × *g* for 2 minutes.

    Alternatively, if desired, an additional particle capture step may be performed using the 0.5ml MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5341) or Maxwell® 16 LEV Magnet (Cat.# AS1261). Transfer the supernatant to a clean tube (not provided). Avoid transferring paramagnetic particles.
15. Remove the cartridges and plungers from the Maxwell® 16 LEV Cartridge Rack, and discard following the recommended guidelines. Do not reuse reagent cartridges, LEV plungers or Elution Tubes.

Storing Eluted RNA

If sample eluates are not processed immediately, the eluted RNA should be stored at –20°C or at –70°C. Consult the protocol for your downstream application for specific storage and handling recommendations.

5.B. Setup for AS3000 Maxwell® 16 MDx Instruments

Refer to the *Maxwell® 16 MDx Instrument Technical Manual* #TM320 for detailed information.

Important: To run the simplyRNA blood protocol, you must have Maxwell® 16 firmware version ≥**1.50** (AS3000) installed on your instrument, and you must use the Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070). Using the original LEV magnetic rod will result in low yields.

1. Turn on the Maxwell® 16 MDx Instrument. The instrument will power up, display the firmware version number, proceed through a self-check and home all moving parts.
2. Verify that the Home screen indicates "LEV" and the LEV hardware is present. Press "Run" to continue.

   Note: Failure to change the Maxwell® 16 Instrument hardware to the LEV configuration could result in instrument damage.
3. Enter user and PIN if this option is enabled.
4. At the Protocols screen, select "RNA," then select "simplyRNA Blood".
5. On the next screen, verify that the correct method and user were chosen. Select "Run/Stop" to continue.
6. Open the door when prompted on the screen, then select "Run/Stop".

Warning: Pinch point hazard.

7. Follow on-screen instructions for bar code reader input if this option is enabled.
8. Transfer the Maxwell® 16 LEV Cartridge Rack containing prepared cartridges on the Maxwell® 16 Instrument platform. Ensure that the rack is placed in the Maxwell® 16 Instrument with the Elution Tubes closest to the door. The rack will only fit in the instrument in this orientation. If you have difficulty fitting the rack on the instrument platform, check that the rack is in the correct orientation. Ensure the rack is level on the instrument platform.

   Note: Hold the Maxwell® 16 LEV Cartridge Rack by the sides to avoid dislodging cartridges from the rack.
9. Verify that samples were added to well #1 of the cartridges, cartridges in the rack are loaded on the instrument, Elution Tubes are present with 50µl of Nuclease-Free Water and LEV Plungers are in well #8. Well #4 should be green to indicate that DNase was added.

Note: Failure to add DNase will result in DNA in the eluate.

5.B. Setup for AS3000 Maxwell® 16 MDx Instruments (continued)

10. Press the Run/Stop button. The platform will retract. Close the door.

Warning: Pinch point hazard.

The Maxwell® 16 MDx Instrument will immediately begin the purification run. The screen will display the approximate time remaining in the run.

Notes:

1. Pressing the Run/Stop button or opening the door will pause the run.
2. If the run is abandoned before completion, the instrument will wash the particles off the plungers and eject the plungers into well #8 of the cartridge. To continue processing the samples, rinse any particles off the plunger into the last well used. Discard the used plungers. Put new plungers into well #8, and start the run from the beginning.

11. When the automated purification run is complete, follow instructions on the screen for data transfer. For detailed instructions, refer to the *Maxwell® 16 MDx Instrument Technical Manual* #TM320 and *Maxwell® Sample Track Software Technical Manual* #TM314.

End of Run

12. Follow the on-screen instructions at the end of the method to open door. Verify that the plungers are located in well #8 of the cartridge at the end of the run. If the plungers are not removed from the magnetic plunger bar, push them down by hand to remove them.
13. Press the Run/Stop button to extend the platform out of the instrument.
14. Remove the Maxwell® 16 LEV Cartridge Rack from the instrument. Remove Elution Tubes containing RNA, and close the tubes.
15. If paramagnetic particles are present in the elution tubes, centrifuge at 10,000 × *g* for 2 minutes.

    Alternatively, if desired, an additional particle capture step may be performed using the 0.5ml MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5341) or Maxwell® 16 LEV Magnet (Cat.# AS1261). Transfer the supernatant to a clean tube (not provided). Avoid transferring paramagnetic particles.
16. Remove the cartridges and plungers from the Maxwell® 16 LEV Cartridge Rack, and discard following the recommended guidelines. Do not reuse reagent cartridges, LEV Plungers or Elution Tubes.

    For the Maxwell® 16 MDx Instrument, ensure samples are removed before the UV light treatment to avoid damage to the nucleic acid.

Storing Eluted RNA

If sample eluates are not processed immediately, the eluted RNA should be stored at –20°C or at –70°C. Consult the protocol for your downstream application for specific storage and handling recommendations.

6. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Causes and Comments
Low RNA yield, RNA degradation or poor reproducibility between samples	Sample contains a low amount of RNA. The amount of RNA present in a sample depends on the metabolic state and white blood cell count.
	The blood sample was too old. Best yields are obtained with fresh blood. Samples that have been stored at 2–10°C for more than 3 days may give reduced yields. Stability of individual messages may vary.
	Use fresh blood, do not freeze blood. For best results, do not freeze the cell pellet. However, the cell pellet resuspended in Homogenization Solution with 1-Thioglycerol may be frozen.
	Lysis Buffer was added to whole blood instead of Cell Lysis Solution. Both red and white blood cells are lysed by Lysis Buffer, so there would be little or no pellet.
	1-Thioglycerol was not added to the Homogenization Solution.
	Lysis Buffer was not added or was added in the wrong order.
	Lysates were not mixed by vortexing long enough.
	The wrong magnet bar was used. Use the High Strength LEV Magnetic Rod and Plunger Bar Adaptor (Cat.# SP1070).
	The wrong method was run with the Maxwell® 16 Instrument.
	RNase introduced by handling. Use sterile, disposable plasticware or baked glassware when handling RNA. Wear clean gloves at all times. RNases introduced during or after purification will degrade the RNA. See Section 7.A, Creating a Ribonuclease-Free Environment.
	All or part of the pellet was lost during removal of the supernatant. Avoid loss of the pellet. Centrifuge for additional time if needed.

6. Troubleshooting (continued)

Symptoms	Causes and Comments
Too many red blood cells in the pellet	The cell pellet may be resuspended a second time with 2ml Cell Lysis Solution (extra not provided) and centrifuged again at 3,000 × *g* for 10 minutes. Cell Lysis Solution may be purchased separately (Cat.# A7933).
Cell pellet in Homogenization Solution is too viscous to pipet	Reduce the viscosity by adding an additional 100µl of Homog enization Solution with 1-Thioglycerol. Vortex or pipet to mix. Add a total of 300µl Lysis Buffer and add all of lysate to the cartridge.
Sample foams during vortexing	Sample will settle during the proteinase K incubation. All liquid and foam can be added to the cartridge. To minimize foaming, the sample can be transferred into a 1.5ml microcentrifuge tube before vortexing.

7. Appendix

7.A. Creating a Ribonuclease-Free Environment

Ribonucleases are extremely difficult to inactivate. Take care to avoid introducing RNase activity into your RNA samples during and after isolation. This is especially important if the starting material was difficult to obtain or is irreplaceable. The following notes may help prevent accidental RNase contamination of your samples.

1. Two of the most common sources of RNase contamination are the user's hands and bacteria or molds that may be present on airborne dust particles. To prevent contamination from these sources, use sterile technique when handling the reagents supplied with this system. Wear gloves at all times. Change gloves whenever ribonucleases may have been contacted.
2. Whenever possible, sterile, disposable plasticware should be used for handling RNA. These materials generally are RNase-free and do not require pretreatment to inactivate RNase.
3. Treat nonsterile glassware, plasticware and electrophoresis chambers before use to ensure that they are RNase-free. Bake glassware at 200°C overnight, and thoroughly rinse plasticware with 0.1N NaOH, 1mM EDTA, followed by RNase-free water. Commercially available RNase removal products also may be used, following the manufacturer's instructions.

   Note: Electrophoresis chambers may be contaminated with ribonucleases, particularly RNase A, from analysis of DNA samples. Whenever possible, set aside a new or decontaminated apparatus for RNA analysis only.
4. Treat solutions not supplied with the system by adding diethyl pyrocarbonate (DEPC) to 0.1% in a fume hood. Incubate overnight with stirring at room temperature in the hood. Autoclave for 30 minutes to remove any trace of DEPC.

   Caution: DEPC is a suspected carcinogen and should only be used in a chemical fume hood. DEPC reacts rapidly with amines and cannot be used to treat Tris buffers.

Note: For all downstream applications, it is essential that you continue to protect your RNA samples from RNases. Continue to wear clean gloves and use solutions and centrifuge tubes that are RNase-free.

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7.B. Related Products

Instrument/Instrument Accessories	Size	Cat.#
Maxwell® 16 Instrument	1 each	AS2000
Maxwell® 16 MDx Instrument	1 each	AS3000
Maxwell® 16 SEV Hardware Kit	1 each	AS1200
Maxwell® 16 LEV Hardware Kit	1 each	AS1250
Maxwell® 16 LEV Cartridge Rack	1 each	AS1251
Maxwell® 16 High Strength LEV Magnetic Rod and Plunger Bar Adaptor	1 each	SP1070

Standard Elution Volume (SEV) Kits	Size	Cat.#
Maxwell® 16 Blood DNA Purification Kit	48 preps	AS1010
Maxwell® 16 Cell DNA Purification Kit	48 preps	AS1020
Maxwell® 16 Tissue DNA Purification Kit	48 preps	AS1030
Maxwell® 16 Total RNA Purification Kit	48 preps	AS1050
Maxwell® 16 Mouse Tail DNA Purification Kit	48 preps	AS1120
DNA IQ™ Reference Sample Kit for Maxwell® 16	48 preps	AS1040
Maxwell® 16 Polyhistidine Protein Purification Kit	48 preps	AS1060

LEV Reagent Kits	Size	Cat.#
Maxwell® 16 simplyRNA Cells Kit	48 preps	AS1270
Maxwell® 16 simplyRNA Tissue Kit	48 preps	AS1280
Maxwell® 16 FFPE Tissue LEV DNA Purification Kit	48 preps	AS1130
Maxwell® 16 FFPE Plus LEV DNA Purification Kit	48 preps	AS1135
Maxwell® 16 Cell LEV DNA Purification Kit	48 preps	AS1140
Maxwell® 16 Viral Total Nucleic Acid Purification Kit	48 preps	AS1150
Maxwell® 16 Tissue LEV Total RNA Purification Kit	48 preps	AS1220
Maxwell® 16 Cell LEV Total RNA Purification Kit	48 preps	AS1225
Maxwell® 16 LEV Blood DNA Kit	48 preps	AS1290
Maxwell® 16 Buccal Swab LEV DNA Purification Kit	48 preps	AS1295
DNA IQ™ Casework Pro Kit for Maxwell® 16*	48 preps	AS1240

*Not For Medical Diagnostic Use.

Accessory Products	Size	Cat.#
Cell Lysis Solution	1L	A7933
MagneSphere® Technology Magnetic Separation Stand (twelve-position)	0.5ml	Z5341
Maxwell® 16 LEV Magnet	1 each	AS1261

[a]U.S. Pat. No. 6,855,499, European Pat. No. 1368629, Japanese Pat. No. 4399164 and other patents pending.

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