Maxwell® RSC miRNA Plasma and Serum Kit

Instructions for Use of Product AS1680.

Promega

Quick Protocol

Solution Preparation and Sample Preprocessing

DNase I Solution

1. Add 275µl of Nuclease-Free Water to the vial of lyophilized DNase I.
2. Invert to rinse DNase off the underside of the cap and swirl gently to mix; do not vortex.
3. Add 5µl of Blue Dye to the reconstituted DNase I as a visual aid for pipetting.
4. Dispense the DNase I Solution into single-use aliquots in nuclease-free tubes. Each purification requires 10µl of DNase I Solution.
5. Store reconstituted DNase I Solution at –30°C to –10°C.
   Note: DNase I Solution maintains activity for up to 10 freeze-thaw cycles.

Preprocessing of Plasma or Serum Samples

1. Use plasma or serum sample volumes between 100–500µl. For plasma or serum samples less than 100µl, add 100µl of Nuclease-Free Water to the sample.
2. Add 80µl of Proteinase K and 230µl of Lysis Buffer C to the plasma or serum sample. Mix by vortexing for 5 seconds.
   Note: Lysis Buffer C contains 1% 1-thioglycerol.
3. Incubate at 37°C for 15 minutes. During this time, prepare the Maxwell® RSC Cartridges.
4. Transfer all of the lysate to well #1 (the largest well in the cartridge) of the Maxwell® RSC Cartridge.
5. Add 10µl of blue DNase I Solution to well #4 of the Maxwell® RSC Cartridge (well #4 contains yellow reagent). After the blue DNase I Solution is added, the reagent in well #4 will be green.
6. Load deck trays onto the instrument and begin the automated purification run.

Preprocessing of Exosome Samples

This kit does not isolate exosomes. For optimal results, do not use more than 75µl of exosomes resuspended in PBS.

1. Up to 200µl of exosome sample in water or non-PBS buffer can be used. For exosome samples less than 200µl, add Nuclease-Free Water to the sample to bring the total volume to approximately 200µl.
   Note: Exosomes may be resuspended in Lysis Bufer C. Refer to the *Maxwell® RSC miRNA Plasma and Serum Kit Technical Manual* #TM546 for more detailed information.
2. Add 80µl of Proteinase K and 230µl of Lysis Buffer C to the exosome sample. Mix by vortexing for 5 seconds.
   Note: Lysis Buffer C contains 1% 1-thioglycerol.
3. Incubate at 37°C for 15 minutes. During this time, prepare the Maxwell® RSC Cartridges.
4. Transfer all of the lysate to well #1 (the largest well in the cartridge) of the Maxwell® RSC Cartridge.
5. Add 10µl of blue DNase I Solution to well #4 of the Maxwell® RSC Cartridge (well #4 contains yellow reagent). After the blue DNase I Solution is added, the reagent in well #4 will be green.
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Maxwell® Method Setup and Cartridge Preparation

Maxwell® Method Setup

Before using the Maxwell® RSC miRNA Plasma and Serum Kit for the first time, the miRNA Plasma and Serum method must be installed on your instrument.

Cartridge Preparation

Prepare cartridges shortly before adding the lysate.

1. Place the cartridges to be used in the deck tray(s) with well #1 (the largest well in the cartridge) 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.
2. Place one plunger into well #8 of each cartridge.
3. Place an empty 0.5ml Elution Tube into the elution tube position for each cartridge in the deck tray.
4. Add 50µl of Nuclease-Free Water to the bottom of each elution tube.

Figure 1. Setup and configuration of the deck tray.

Notes:

1. Clean specimen or reagent spills on any part of the deck tray with a detergent-water solution, followed by a bacteriocidal spray or wipe, and then water. Do not use bleach on any instrument parts.
2. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with supported Maxwell® Instruments.

Maxwell® Instrument Run

1. Follow the instrument run instructions in the *Maxwell® RSC miRNA Plasma and Serum Kit Technical Manual* #TM546.

Additional protocol information is in Technical Manual #TM546, available online at: **www.promega.com**

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