

Maxwell® RSC PureFood Pathogen Kit

Instructions for Use of Product AS1660.

Preparing Food Samples for DNA Purification

This Quick Protocol provides instructions for use of the Maxwell® RSC PureFood Pathogen Kit with the Maxwell® RSC Instrument to purify pathogen DNA from food samples. For detailed instructions, including information on instrument setup and troubleshooting, please refer to the *Maxwell® RSC PureFood Pathogen Kit Technical Manual #TM528*, available at:

www.promega.com/protocols/

Materials to Be Supplied by the User

- microcentrifuge tubes, 1.5ml or 2.0ml
- sterile, aerosol-resistant pipette tips
- ThermoMixer® heat block
- Stomacher® lab paddle or vortexer
- Stomacher® bags
- 25g of food sample

Preparing Samples with Stomacher® Lab Paddle

This preprocessing protocol requires a mechanical mixing device such as a Stomacher® lab paddle or vortexer for homogenization of food samples. Follow the manufacturer's recommendation for processing the sample.

1. Weigh and add 25g of food sample into a filtered Stomacher® bag.
2. Add 225ml of pre-warmed select enrichment broth to Stomacher® bag.
3. If necessary, add select bacteria to the Stomacher® bag.
4. Place Stomacher® bag in Stomacher® lab paddle and select to mix at 230rpm for 30–60 seconds.
5. Incubate at 37°C for 20–24 hours.

Bacterial Sample Lysis from Food Matrix

1. Add 800µl of food/bacterial sample to a microcentrifuge tube.
2. Add 200µl of Lysis Buffer A to each tube.
3. Incubate at 56°C for 4 minutes in a ThermoMixer® Device, shaking at 500–1,000rpm.
4. Prepare RSC cartridges as described in the Preparing the Cartridge section during the incubation.
5. Add 300µl of Lysis Buffer to each tube.
6. Mix by vortexing for 5–10 seconds.
7. Add the entire sample to well #1 of the cartridge.
8. Proceed to Running the Method on the Maxwell® RSC Instrument (Cat.# AS4500) for DNA purification.

Maxwell® RSC PureFood Pathogen Kit

Instructions for Use of Product AS1660.

Automated DNA Purification

Preparing the Cartridge

1. Place the cartridges to be used in the Maxwell® RSC deck tray with the labeled side facing away from the Elution Tube.
2. 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 cartridge rack.

3. Place a Maxwell® RSC Plunger in well #8 of each cartridge. Well #8 is the well closest to the Elution Tube.

Note: Use only the plungers provided in the Maxwell® RSC PureFood Pathogen Kit.

4. Place 0.5ml Elution Tubes in the front of the deck tray. Add 50µl of Elution Buffer to the bottom of each Elution Tube.



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

Notes:

- a. If Elution Buffer is on the side of the tube, the elution may be suboptimal.
- b. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may not work with the Maxwell® RSC Instrument.

Running the Method on the Maxwell® RSC Instrument (Cat.# AS4500)

1. Refer to the *Maxwell® RSC Instrument Operating Manual* #TM411 for detailed information on running methods. To run the PureFood Pathogen protocol, the Maxwell® RSC PureFood Pathogen method must be installed on your instrument. The method is available at: www.promega.com/resources/tools/maxwellrscmethod/. See the *Maxwell® RSC Methods Installation Technical Manual* #TM435 for instructions.
2. Follow the instrument run instructions in the *Maxwell® RSC PureFood Pathogen Kit Technical Manual* #TM528.

Additional protocol information in Technical Manual #TM528, available online at: www.promega.com