



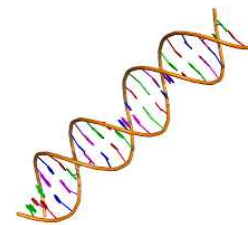
Nov 24, 2025

Version 2

DNA Extraction from inoculated broth for metagenomics using the Promega Maxwell RSC cultured kit V.2



Version 1 is forked from [DNA Extraction from Inoculated Broth for WGS using the Maxwell RSC Cultured Cells DNA Kit](#)



DOI

<https://dx.doi.org/10.17504/protocols.io.6qpvrqb72lmk/v2>

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Protocol Citation: Christopher Duda, Anna Brover, Padmini Ramachandran, Christopher Grim 2025. DNA Extraction from inoculated broth for metagenomics using the Promega Maxwell RSC cultured kit. **protocols.io**

<https://dx.doi.org/10.17504/protocols.io.6qpvrqb72lmk/v2> Version created by **Christopher Duda**

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Protocol status: Working

We use this protocol and it's working

Created: November 24, 2025

Last Modified: November 24, 2025

Protocol Integer ID: 233380

Keywords: DNA Extraction, Automation, broth for metagenomic, metagenomic, extracting dna, metagenomic enrichment, dna extraction, dna from gram, promega maxwell rsc instrument, promega maxwell rsc, selective enrichment broth, positive bacterial cells from pure culture, genomic dna, universal enrichment broth, maxwell rsc, maxwell rsc 48 instrument, dna, inoculated broth, positive bacterial cell, extraction method purify, extraction, purification process, information for extraction

Abstract

The Maxwell® RSC Cultured cells DNA Kit is used with the Maxwell RSC or Maxwell RSC 48 Instrument from Promega. This extraction method purifies genomic DNA (gDNA) from pure culture and/or from metagenomic enrichments efficiently using automation. The kit utilizes a silica-based paramagnetic particle that is used during the purification process.

This protocol will provide information for extraction from universal enrichment broth or selective enrichment broth. Additional steps are performed when extracting DNA from gram-positive organisms.

This SOP is applicable to all laboratories with the intent of obtaining high quality, purified genomic DNA or quasi-metagenomic DNA through the use of a Promega Maxwell RSC Instrument. DNA can be isolated from both gram-negative or gram-positive bacterial cells from pure culture or enrichments for use in subsequent sequencing protocols.

Guidelines

Table 1. Supported Instruments.

Instrument	Cat.#	Technical Manual
Maxwell® RSC	AS4500	TM411
Maxwell® RSC 48	AS8500	TM510
Maxwell® CSC RUO Mode	AS6000	TM573
Maxwell® CSC 48 RUO Mode	AS8000	TM628
Maxprep™ Liquid Handler	AS9100, AS9101, AS9200, AS9201	TM509

PRODUCT	SIZE	CAT.#
Maxwell® RSC Cultured Cells DNA Kit	48 preps	AS1620

For Research Use Only. Not for use in diagnostic procedures. Sufficient for 48 automated isolations from samples containing up to 5×10^6 tissue culture cells or up to 2×10^9 bacterial cells. Cartridges are for single use only. Includes:

- 48 Maxwell® RSC Cartridge (RSCI)
- 1 Maxwell® RSC Plunger Pack (48 Plungers)
- 50 Elution Tubes (0.5ml)
- 20ml Elution Buffer

Storage Conditions: Store the Maxwell® RSC Cultured Cells DNA Kit at +15°C to +30°C.



Materials

Reagents:

Maxwell[®] RSC Cultured Cells DNA Kit -- Cat# AS1620 [Promega]

DNase free, RNase free, molecular grade water -- Cat# 10977015 [Invitrogen] *or equivalent*

PureLink[™] RNase A (20 mg/ml) -- Cat# 12091021 [ThermoFisher Scientific] *or equivalent*

TE, pH 7.0, RNase-free -- Cat# AM9861 [ThermoFisher Scientific] *or equivalent*

Lysozyme -- Cat# L4919 [Sigma Aldrich] *or equivalent*

Metapolyzyme [https://www.sigmaaldrich.com/US/en/product/sigma/mac4l?](https://www.sigmaaldrich.com/US/en/product/sigma/mac4l?srsId=AfmBOopn8iMZxurbqDXFPbwvM9WQfM9RHM5qPgtAHS0Acn0EvNobDGT2)

[srsId=AfmBOopn8iMZxurbqDXFPbwvM9WQfM9RHM5qPgtAHS0Acn0EvNobDGT2](https://www.sigmaaldrich.com/US/en/product/sigma/mac4l?srsId=AfmBOopn8iMZxurbqDXFPbwvM9WQfM9RHM5qPgtAHS0Acn0EvNobDGT2) for metagenomic DNA extraction from enrichments

Equipment:

Maxwell[®] RSC or Maxwell[®] RSC 48 Instrument

Pipettors

Centrifuge

Incubator

Heatblock

Vortexer

Consumables:

2.0 ml Eppendorf Safe-Lock Tubes -- Cat# 05-402-7 [ThermoFisher Scientific]

1.5 ml Eppendorf DNA LoBind Microcentrifuge Tubes -- Cat# 13-698-791 [ThermoFisher Scientific]

Pipette Tips

Safety warnings



Safety Information: The Maxwell® RSC Cartridges contain ethanol, isopropanol and guanidine thiocyanate. Ethanol and isopropanol should be considered flammable, harmful and irritants. Guanidine thiocyanate should be considered toxic, harmful and an irritant. Refer to the SDS for detailed safety information.



Samples used with the Maxwell® RSC Cartridges may contain potentially infectious substances. Wear appropriate protection (e.g., gloves and safety glasses) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances when used with this system.



Caution: Handle cartridges with care; seal edges may be sharp. Bleach reacts with guanidine thiocyanate and should not be added to any sample waste from these cartridges.

Refer to the full SDS for detailed safety information.



AS1620-en-US.pdf 369.7KB

Pre-processing of Culture Enrichments for Gram Positive Communities

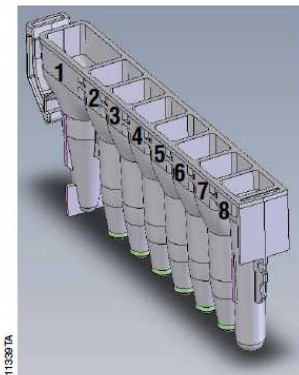
- 1 Due to the thick peptidoglycan layer of **Gram-Positive** bacteria extra lysing is necessary. Follow the steps below for this pre-processing. While we show 2 different lysis methods, ***DO ONLY step 3 OR 4***
- 2
 1. Spin **enrichment cultures** down at 3,000 rcf for 20 minutes.
 2. Remove and discard the supernatant from the tube.
 3. Continue onto **EITHER Step 3 or 4.**
- 3 **Lysis With LE-Lysozyme *Only do one lysis step***
 1. Resuspend the pellet with 400 µl of the TE-Lysozyme buffer.
 2. Vortex for 5-10 seconds
 3. Incubate at 37 °C for at least 30 minutes. If the enrichment contains a highly gram-positive community, an increased lysis time may be necessary.
 4. Add 400 µL of the lysate to the first well of the maxwell kit cartridge.
 5. Proceed to **Cultured Cells DNA Cartridge Preparation**
- 4 **Lysis With Metapolyzyme *Only do one lysis step***
 1. Resuspend the pellet with 50ul of metapolyzyme (Sigma-aldrich).
 2. Vortex for 5-10 seconds
 3. Incubate at 37 °C for at least 30 minutes. If the enrichment contains a highly gram-positive community, an increased lysis time may be necessary.
 4. Add 400 µL of the lysate to the first well of the maxwell kit cartridge.
 5. Proceed to **Cultured Cells DNA Cartridge Preparation**

Loading Enrichments into Maxwell Cartridges

- 5 Transfer **at least 8 mL of Rappaport-Vassiliadis Broth (RV) or Tetrathionate broth (TT) cultures** or overnight general enrichment (mBPW, UPB, TSB, APW) to a 15 mL centrifuge tube
- 6
 1. For **RV and/or general enrichment**, spin cultures down at 3,000 rcf for 20 minutes.
 2. Remove and discard the supernatant from the tube.
 3. Using a Maxwell Promega DNA isolation kit cartridge, remove 400 µL of the lysis buffer (well 1, see step 4 for details) and resuspend cells by vortexing.
 5. Add the lysis buffer and cell solution back to the first well of the maxwell kit cartridge.
- 7
 1. For **TT**, Spin cultures down at 3,000 rcf for 20 minutes
 2. Remove and discard the supernatant from the tube.
 3. Using a Maxwell Promega DNA isolation kit cartridge, remove 400 µL of the lysis buffer (well 1, See step 4 for details) and resuspend by vortexing.
 5. Spin down tubes at 300 rcf for 3 minutes to remove solid salts from the supernatant.
 6. Add the lysis buffer and cell solution back to the first well of the maxwell kit cartridge.

Cultured Cells DNA Kit Cartridge Preparation

- 8
 1. Turn on the instrument and associated PC. Start the RSC software and wait for the instrument to initialize.
 2. Open the door of the instrument by pressing the **Door** icon at the top right corner of the software.
 3. Remove the deck tray(s) from the instrument. Press the **Door** icon to close the instrument door.
 4. Place the number of cartridges needed for the number of samples into the deck tray. The cartridges are inserted with **Well 1 (lysis buffer)** at the top of the tray. Figure 1 illustrates the well locations on the cartridge. Press down on the cartridge to snap it into position.
 5. Carefully peel back the seal on the cartridges. Ensure that the entire seal is removed.
 6. Add a plunger to **Well 8** in each cartridge.



User Adds to Wells

1. Cultured Cell sample (up to 5×10^6 tissue culture cells; up to 2×10^9 bacterial cells)
8. RSC Plunger

Figure 1. Cartridge setup

7. Place elution tubes (provided with kit) into the elution tube position with the lid facing the bottom of the deck tray (Figure 2). Add 100 μ l of **Elution buffer** to each tube. Ensure there are no bubbles in the tubes, and the buffer is at the bottom of each tube.



Figure 2. Elution Buffer Tube and Lid positioning

8. Transfer 400 μ l of the sample culture to the lysis buffer in Well 1 of the corresponding cartridge and pipette gently up and down 10 times to mix. Proceed to the Instrument Setup and Extraction Run step.

9. Optional: RNase A Treatment

- a. Allow cells to lyse for 10 minutes in **Well 1**.
- b. Add 2 μ l of RNase A to each sample in **Well 1**. Pipette up and down to mix.
- c. Incubate for an additional 10 minutes prior to starting the instrument.

Instrument Setup and Extraction Run

- 9 1. In the RSC Software, press **Start** to access the extraction method selection screen.
2. Select **Cultured Cells DNA** method, then press **Proceed**.
3. Select the lanes being used by touching them. Sample names can be inputted at this time.
Note: For the RSC48, press the "BACK" and "FRONT" buttons on the bottom of the screen to move to each respective rack.
4. Press **Proceed** to continue. The instrument door will open.

5. Install the deck tray(s) into the platform by angling the back of the tray and inserting first. Press down the front of the tray until it snaps into place. Ensure the deck tray is level and fully seated.

Note: For the RSC48, ensure the tray labelled FRONT and BACK are placed in the correct places in the instrument.

6. Verify that all of the cartridges are properly inserted into the tray, the elution tubes are present and uncapped, and then plungers are in **Well 8**.

Note: Ensure there are no old plungers left on the instrument from a prior run.

7. Press **Start** to begin the extraction run.

8. Follow on-screen instructions at the end of the run.

9. Remove the deck tray(s) from the instrument. The DNA can be transferred to 1.5 ml LoBind Tubes for storage.

10. Remove the cartridges and plungers from the deck trays and discard in a biohazard container.

11. Clean the tray with 70% Ethanol. **Do NOT use bleach to clean the trays as this reacts with Guanidine Thiocyanate.**

12. Proceed to the **Cleaning and Decontamination** steps.

13. Proceed to quantify the DNA using the **DNA Quantification using the Qubit Fluorometer** SOP.

Cleaning and Decontamination

10 The Maxwell RSC instrument should be wiped down with 70% Ethanol as needed.

Follow up each extraction with the **Sterilization** process on the instrument.