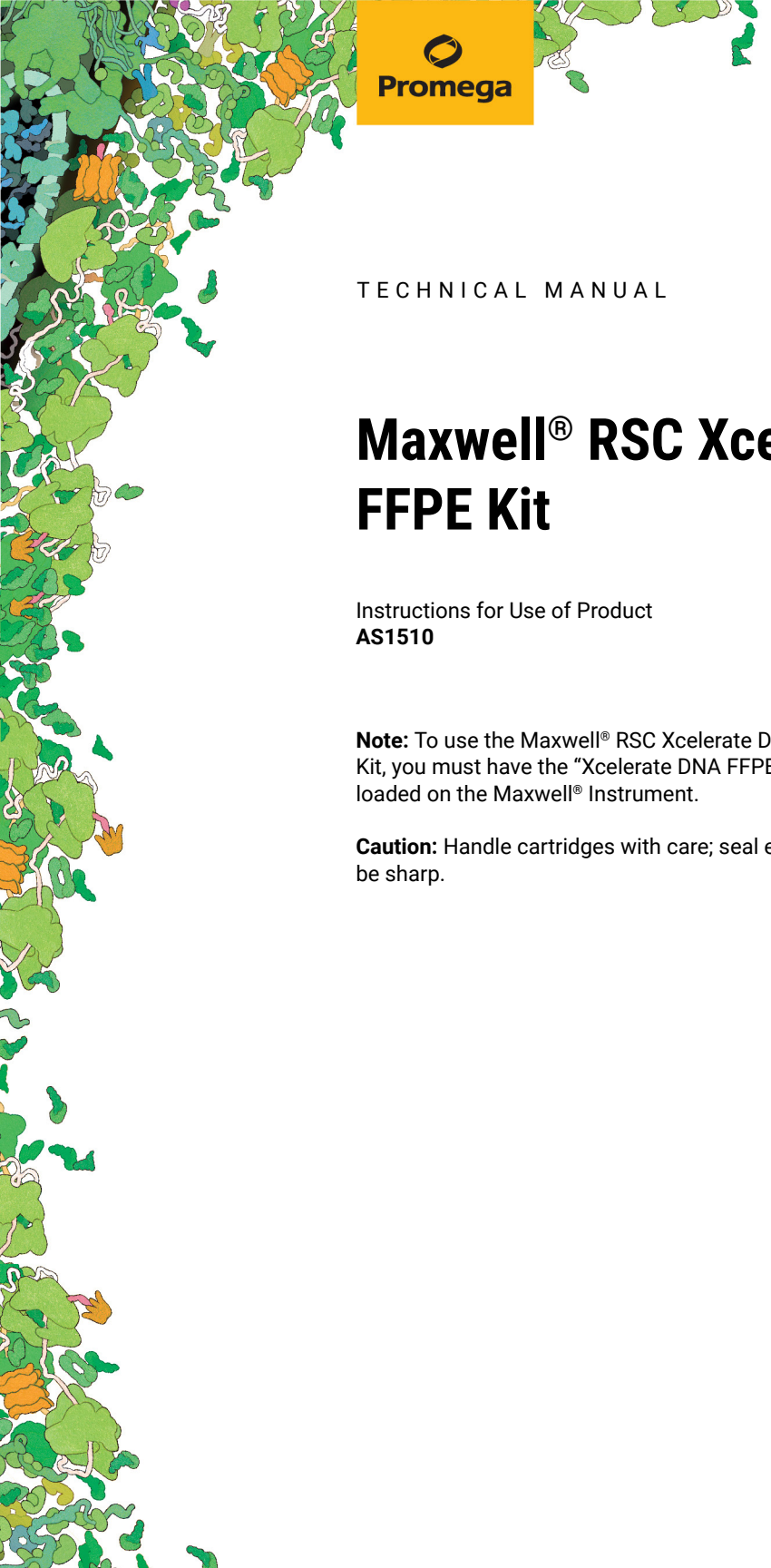

TECHNICAL MANUAL

Maxwell® RSC Xcelerate DNA FFPE Kit

Instructions for Use of Product
AS1510

Note: To use the Maxwell® RSC Xcelerate DNA FFPE Kit, you must have the "Xcelerate DNA FFPE" method loaded on the Maxwell® Instrument.

Caution: Handle cartridges with care; seal edges may be sharp.

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Maxwell® RSC Xcelerate DNA FFPE Kit

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

1. Description

The Maxwell® RSC Xcelerate DNA FFPE Kit[a] is used in combination with the Maxwell® Instruments specified in Table 1 to provide a simple method for efficient, automated purification of genomic DNA (gDNA) from formalin-fixed, paraffin-embedded (FFPE) mammalian tissue samples. The Maxwell® Instruments are designed for use with the predispensed reagent cartridges and additional reagents supplied in the kit, with preprogrammed purification methods, thereby maximizing simplicity and convenience. The Maxwell® Instruments can process from one to the maximum sample number in approximately 40 minutes. The purified gDNA can be used directly in downstream amplification-based assays such as PCR.

Table 1. Supported Instruments.

Instrument	Cat.#	Operating Manual
Maxwell® RSC	AS4500	TM411
Maxwell® RSC 48	AS8500	TM510
Maxwell® CSC RUO Mode	AS6000	TM573
Maxwell® CSC 48 RUO Mode	AS8000	TM628

The Maxwell® RSC Xcelerate DNA FFPE Kit purifies nucleic acid using paramagnetic particles, which provide a mobile solid phase to optimize sample capture, washing and purification of gDNA. The Maxwell® Instruments are magnetic particle-handling instruments that efficiently bind gDNA to the paramagnetic particles in the first well of a prefilled cartridge. The samples are processed through several washes before the gDNA is eluted.

The Maxwell® RSC Xcelerate DNA FFPE Kit has been designed to achieve gDNA purification from FFPE tissue samples using shortened preprocessing times. The kit includes a formulated chemistry [Xcelerate Buffer (XB1)] that catalyzes the reversal of DNA-protein and DNA-DNA formalin crosslinks.

Sample Considerations: DNA purification from FFPE tissue samples can be challenging due to tissue characteristics such as fibrosity, lipid composition, nuclease levels and the cell number available in the tissue section. In addition, variability in how the tissue is handled prior to and during fixation, including the duration for which the tissue is exposed to formalin during the tissue fixation process, greatly influences the degree of crosslinking and fragmentation of nucleic acids in the FFPE tissue. All these attributes may influence the quality and the amount of amplifiable nucleic acids that can be purified from FFPE tissue sections. During development, the Maxwell® RSC Xcelerate DNA FFPE Kit was evaluated with a variety of human FFPE tissue types and formats (e.g., FFPE tissue sections on slides versus curls) to ensure optimal purification of the available amplifiable DNA.

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT.#
Maxwell® RSC Xcelerate DNA FFPE Kit	**48 preps**	**AS1510**

For Research Use Only. Not for use in diagnostic procedures. Sufficient for 48 automated isolations from FFPE tissue samples. Cartridges are single-use only. Includes:

- 25ml Mineral Oil
- 20ml Lysis Buffer
- 2 × 1ml Proteinase K Solution
- 100µl Blue Dye
- 1ml RNase A Solution
- 48 Maxwell® FFPE Cartridges
- 1 Maxwell® RSC Plunger Pack (48 plungers)
- 50 Elution Tubes (0.5ml)
- 25ml Nuclease-Free Water
- 2 × 700µl Xcelerate Buffer (XB1)

Storage Conditions: Store the Maxwell® RSC Xcelerate DNA FFPE Kit at ambient temperature (+15 to +30°C).

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

Maxwell® FFPE Cartridges are designed to be used with potentially infectious substances. Wear protection (e.g., gloves and goggles) 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.

3. Sample Preparation

Materials to Be Supplied By the User

- microcentrifuge
- 1.5–2.0ml tubes for incubation of samples (e.g., Microtubes, 1.5ml; Cat.# V1231)
- FFPE tissue sections up to a total input volume of 2.0mm^3
  Note: Store samples at room temperature (15–30°C).
- razor blades (**Note:** Use caution when using razor blades to scrape samples from slides.)

3.A. Sample Information

The Maxwell® RSC Xcelerate DNA FFPE Kit is only intended for use with FFPE tissue samples. It is not intended for use with nonFFPE tissue samples, such as fresh or frozen tissue samples.

The Maxwell® RSC Xcelerate DNA FFPE kit performance was evaluated with FFPE tissue samples prepared with 10% neutral-buffered formalin.

The Maxwell® RSC Xcelerate DNA FFPE Kit performance was evaluated by isolating DNA from FFPE mammalian (human) tissue input volume of 0.02–2.0mm^3.

3.B. Preparing FFPE Tissue Samples

Place the FFPE tissue section into a 1.5ml or 2.0ml microcentrifuge tube. If using slide-mounted tissue sections, scrape section off the slide using a clean razor blade. Centrifuge the tube at maximum speed for 15 seconds to collect the sample at the bottom of the tube, if necessary.

Note: FFPE tissue sections with a total volume of up to 2mm^3 can be used.

4. Manual Preprocessing

4.A. Preprocessing FFPE Tissue Section Samples

Materials to Be Supplied by the User

- microcentrifuge
- pipettors and pipette tips for sample transfer into prefilled reagent cartridges
- heating blocks set at 56°C and 80°C

1. Add 300µl of Mineral Oil to the sample tubes. Vortex for 10 seconds.
2. Heat the samples at 80°C for 2 minutes. Place the samples at room temperature while the master mix is prepared.

3. Prepare a master mix of the Lysis Buffer, Proteinase K Solution and Blue Dye as shown in the table:

Reagent	Amount per Reaction	Reactions (Sample Number + 2)	Total
Lysis Buffer	224µl	n + 2	224 × (n + 2)µl
Proteinase K	25µl	n + 2	25 × (n + 2)µl
Blue Dye	1µl	n + 2	1 × (n + 2)µl

For fewer than six samples, prepare enough master mix for n + 1 samples.

Note: Use the master mix within 1 hour. Do not store master mix for later use.

4. Add 250µl of master mix to each sample tube, and vortex for 5 seconds.
5. Centrifuge at 10,000 × *g* for 20 seconds to separate layers. If a pellet is present in the aqueous layer (lower, blue layer), gently mix pellet and aqueous phase with a pipette.
6. Transfer the sample tubes to a 56°C heating block and incubate for 15 minutes.
7. Remove the sample tubes from the heating block. Proceed immediately to Step 8.
8. Add 25µl of Xcelerate Buffer (XB1) to the aqueous layer (lower, blue layer). Gently mix Xcelerate Buffer (XB1) into the lysate by pipetting. Mixing the lower aqueous layer (blue) and the Xcelerate Buffer (XB1; yellow) will result in a green color, indicating the Xcelerate Buffer (XB1) has been added and is sufficiently mixed.

   Note: Add exactly 25µl of the Xcelerate Buffer (XB1). Adding more or less of the Xcelerate Buffer (XB1) may negatively affect DNA yields.
9. Transfer the sample tube to an 80°C heating block and incubate for 30 minutes.

   Note: Incubate the samples for exactly 30 minutes. Incubating for longer or shorter times may negatively affect DNA yields.
10. Remove the sample tubes from the heating block, and cool the samples to room temperature for 5 minutes.
11. Add 10µl of RNase A to the aqueous (green) phase in each sample tube. Mix by pipetting.
12. Incubate sample tubes for 5 minutes at room temperature (15–30°C). During the incubation, begin cartridge preparation (see Section 4.B).
13. Centrifuge the sample tubes at full speed in a microcentrifuge for 5 minutes.
14. Immediately transfer the green aqueous phase to well #1 of a Maxwell® FFPE Cartridge.

    Note: Take care to avoid the pellet and any insoluble tissue debris. Transfer of insoluble tissue into the well #1 of the cartridge may negatively affect DNA yields.

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4.B. Manually Preparing the Maxwell® FFPE Cartridge

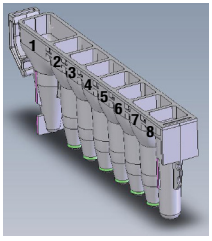
1. Change gloves before handling Maxwell® FFPE Cartridges, RSC Plungers and Elution Tubes (0.5ml). Place the cartridges to be used in the deck tray(s) with well #1 (the largest well in the cartridge) facing away from the elution tubes. Press down on the cartridge to snap it into position. Carefully peel 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 elution tube into the elution tube position for each cartridge in the deck tray(s).
4. Add 50µl of Nuclease-Free Water to the bottom of each elution tube.

   Note: Only use the Nuclease-Free Water provided in the Maxwell® RSC Xcelerate DNA FFPE Kit. Using other elution buffers can affect DNA purification.
5. Proceed to Section 6, Maxwell® Instrument Setup and Run.

   Notes:

   a. Specimen or reagent spills on any part of the deck tray should be cleaned with a detergent-water solution, followed by a bacteriocidal spray or wipe and then water. Do not use bleach on instrument parts.

   b. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell® Instrument.



User Adds to Wells

1. Sample lysates

8. RSC Plunger

Figure 1. Maxwell® FFPE Cartridge contents.

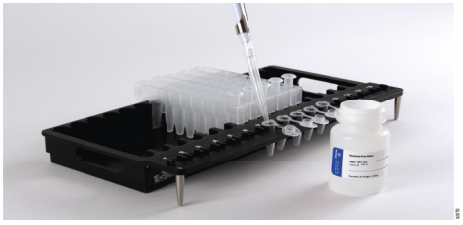


Figure 2. Setup and configuration of the deck trays. Nuclease-Free Water is added to the elution tubes as shown. Plungers are in well #8 of the cartridge.

5. Maxwell® Instrument Setup and Run

Refer to the *Maxwell® RSC Instrument Operating Manual* #TM411 or *Maxwell® RSC 48 Instrument Operating Manual* #TM510 for detailed information.

1. Turn on the Maxwell® Instrument and Tablet PC. Sign in to the Tablet PC, and start the Maxwell® software by double-touching the icon on the desktop. The instrument will power up, proceed through a self test and home all moving parts.
2. Touch **Start** to access the 'Methods' screen.
3. On the 'Methods' screen, select a method using one of the two options below:
   a. Touch the Xcelerate DNA FFPE method.
   b. Use a bar code reader to scan the 2D bar code on the kit box to automatically select the appropriate method.
4. Verify that the Xcelerate DNA FFPE method has been selected, and touch the **Proceed** button. If requested by the software, enter any kit lot and expiration information required by the Administrator.
5. On the 'Cartridge Setup' screen (if shown), touch the cartridge positions to select/deselect any positions to be used for this extraction run. Enter any required sample tracking information, and touch the **Proceed** button to continue.

   Note: When using the Maxwell® RSC 48 Instrument, use the **Front** and **Back** buttons to select/deselect cartridge positions on each deck tray.
6. Open the door and confirm that all Extraction Checklist items have been performed. Verify that samples were added to well #1 of the cartridges, cartridges are loaded onto the deck tray(s), uncapped elution tubes are present with Elution Buffer and plungers are in well #8. Transfer the deck tray(s) containing the prepared cartridges onto the Maxwell® Instrument platform.

   Inserting the Maxwell® Deck Tray: Hold the deck tray by the sides to avoid dislodging cartridges from the deck tray. Ensure that the deck tray is placed in the Maxwell® Instrument with the elution tubes closest to the door. Angle the back of the deck tray downward and place into the instrument so that the back of the deck tray is against the back of the instrument platform. Press down on the front of the deck tray to firmly seat the deck tray on the instrument platform. If you have difficulty fitting the deck tray on the platform, check that the deck tray is in the correct orientation. Ensure the deck tray is level on the instrument platform and fully seated.

   Note: When using the Maxwell® RSC 48 Instrument, check the identifier on the Maxwell® RSC 48 Deck Tray to determine whether it should be placed in the front or back of the instrument.

5. Maxwell® Instrument Setup and Run (continued)

7. Touch the **Start** button to begin the extraction run. The platform will retract, and the door will close.

Warning: Pinch point hazard.

Note: If using a 48-position Maxwell® instrument and the Vision System has been enabled, the deck tray(s) will be scanned as the door retracts. Any errors in deck tray setup (e.g., plungers not in well #8, elution tubes not present and open) will cause the software to return to the 'Cartridge Setup' screen, and problem positions will be marked with an exclamation point in a red circle. Touch the exclamation point for a description of the error and resolve all error states. Touch the **Start** button again to repeat deck tray scanning and begin the extraction run.

8. The Maxwell® Instrument will immediately begin the purification run. The screen will display information including the user who started the run, the current method step being performed and the approximate time remaining in the run.

   Notes:

   a. Touching the **Abort** button will abandon the run. All samples from an aborted run will be lost.

   b. If the run is abandoned before completion, you may be prompted to check whether plungers are still loaded on the plunger bar. If plungers are present on the plunger bar, you should perform Clean Up when requested. If plungers are not present on the plunger bar, you can choose to skip Clean Up when requested. The samples will be lost.

9. When the run is complete, the user interface will display a message that the method has ended.

End of Run

10. Follow on-screen instructions at the end of the method to open the door. Verify that plungers are located in well #8 of the cartridge at the end of the run. If plungers are not removed from the plunger bar, follow the instructions in the *Maxwell® RSC Instrument Operating Manual* or the *Maxwell® RSC 48 Instrument Operating Manual* to perform a Clean Up process to attempt to unload the plungers.

11. Remove the deck tray(s) from the instrument. Remove elution tubes containing DNA, and cap the tubes. If paramagnetic particles are present in the elution tubes, centrifuge at 10,000–20,000 × *g* for 2–5 minutes. After the run is complete, the extraction run report will be displayed. From the 'Report View' screen, you can print or export this report or both.

12. Remove the cartridges and plungers from the deck tray(s), and discard as hazardous waste following your institution's recommended guidelines. Do not reuse reagent cartridges, plungers or elution tubes.

Note: Ensure samples are removed before performing any required UV light treatment to avoid damage to the nucleic acid.

6. Quantitation Recommendations

Determine whether the purified DNA sample yield and purity meets the input requirements for the appropriate downstream assay prior to use in that assay. Kit performance was evaluated based upon the purification of amplifiable DNA. Other means of quantitation, including absorbance or fluorescent dye binding, may not correlate with amplification (1). Absorbance readings for purified FFPE tissue samples may overestimate yield; we recommend using other methods for determining yield (1).

7. Troubleshooting

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

Symptoms	Causes and Comments
Lower than expected concentration of DNA in eluate	Kit performance has been evaluated by isolating DNA from from FFPE tissue samples up to 2.0mm^3. It is not designed for sample volumes larger than 2.0mm^3. Only use sections that meet the size specification. (A typical FFPE tissue section should yield amplifiable DNA depending on tissue size, cellularity, formalin fixation condition and handling.)
	The kit is intended for use with FFPE mammalian tissue samples. It is not intended for use with nonFFPE tissue samples, such as fresh or frozen tissue samples or with FFPE tissue samples collected from nonmammalian tissues.
	The kit performance was evaluated with FFPE tissue samples prepared with 10% neutral-buffered formalin. Repeat the purification with FFPE tissue sections prepared with 10% neutral-buffered formalin.
	The kit performance was not evaluated with stained FFPE tissue curls or sections. Repeat the purification with unstained FFPE tissue curl or section.
	Kit performance was evaluated based upon the purification of amplifiable DNA. Other means of quantitation, including absorbance or fluorescent dye binding, may not correlate with amplification. Use an amplification quantitation method to assess yield.
	Insoluble tissue debris can negatively affect DNA binding in the Maxwell® cartridge. Ensure that the centrifugation step sufficiently pellets tissue debris, and avoid the pellet when using a pipette to transfer the lysate to well #1 of the cartridge.
	For some FFPE tissue samples, using the Xcelerate Buffer (XB1) solution may not be optimal, and a longer decrosslinking incubation without using the Xcelerate Buffer (XB1) solution may be effective. In this case, do not add the Xcelerate Buffer (XB1) solution (Step 8 in Section 4.A). After Step 6 in Section 4.A, proceed to the decrosslinking incubation during Step 9 (Section 4.A), and incubate for 4 hours at 80°C or overnight (14–18 hours) at 70°C.

7. Troubleshooting (continued)

Symptoms	Causes and Comments
Lower than expected quality (the eluate contains highly fragmented DNA or inhibitors of downstream assays)	Formalin fixation and subsequent crosslink reversal will fragment DNA. If the DNA is fragmented prior to extraction and purification, fragmented DNA will be purified with this kit. Repeat purification with an adjacent section to assess whether the fragmentation is inherent to the sample or if the DNA is fragmented during the purification process.
	Extended decrosslinking incubations can fragment FFPE tissue DNA. Ensure that Step 9 in Section 4.A is being performed at 80°C for no longer than 30 minutes. If higher molecular weight DNA is desired, Step 9 in Section 4.A can be performed at 80°C for 20 minutes or 25 minutes, but amplifiable yields may be lower.
	Some amplification assays are particularly sensitive to the presence of inhibitors. Downstream assay controls should identify the presence of an amplification inhibitor in the eluate. The user is responsible for verifying the compatibility of this product with downstream assays.

8. Reference

1. Bonin, S. *et al*. (2010) Multicentre validation study of nucleic acids extraction from FFPE tissues. *Virchows Arch.* **457**, 309–17.

9. Related Products

Instrument and Accessories

Product	Size	Cat.#
Maxwell® RSC Instrument	1 each	AS4500
Maxwell® RSC 48 Instrument	1 each	AS8500
Maxwell® RSC Plunger Pack	48/pack	AS1670
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
Maxwell® RSC/CSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC/CSC 48 Back Deck Tray	1 each	AS8402

Maxwell® RSC Reagent Kits

For a list of available Maxwell® RSC purification kits, visit: **www.promega.com**

[a]U.S. Pat. No. 7,329,488 and Korean Pat. No. 10-0483684.

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