TECHNICAL MANUAL

Maxwell® CSC DNA FFPE Kit

Instructions for Use of Product
AS1350

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

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INSTRUCTIONS FOR
USE OF PRODUCT
AS1350

IVD

Revised 3/21
TM395

Maxwell® CSC DNA FFPE 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

The Maxwell® CSC DNA FFPE Kit is only available in certain countries. This product meets the essential requirements of EU Directive 98/79/EC on in vitro diagnostic medical devices.

1. Description

The Maxwell® CSC DNA FFPE Kit[a] is used in combination with the Maxwell® Instruments specified in Table 1 to provide an easy method for efficient, automated purification of genomic DNA (gDNA) from FFPE (formalin-fixed, paraffin-embedded) tissue samples. The Maxwell® CSC 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® CSC Instruments can process from one to the maximum number of samples allowed in approximately 45 minutes, and the purified DNA can be used directly in downstream amplification-based assays such as PCR.

Table 1. Supported Instruments

Instrument	Cat.#	Technical Manual
Maxwell® CSC	AS6000	TM457
Maxwell® CSC 48	AS8000	TM623

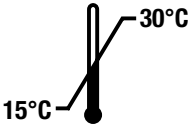
1. Description (continued)

The Maxwell® CSC 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® CSC Instruments are magnetic particle-handling instruments. This system allows efficient binding of gDNA to the paramagnetic particles in the first well of a prefilled cartridge and moves the sample through the wells of the cartridge. This approach to magnetic capture avoids common problems such as clogged tips or partial reagent transfers, which result in suboptimal purification processing by other commonly used automated systems.

2. Product Components, Storage Conditions and Symbols Key

PRODUCT	SIZE	CAT.#
Maxwell® CSC DNA FFPE Kit	**48 preps**	**AS1350**

For In Vitro Diagnostic Use. Professional use only. Sufficient for 48 automated isolations from FFPE samples. The Maxwell® FFPE Cartridges are for single use only.



Includes:

- 25ml Mineral Oil
- 20ml Lysis Buffer
- 2 × 1ml Proteinase K (PK)
- 100µl Blue Dye
- 1ml RNase A
- 48 Maxwell® FFPE Cartridges
- 50 CSC/RSC Plungers
- 50 Elution Tubes (0.5ml)
- 25ml Nuclease-Free Water

Storage Conditions: Store the Maxwell® CSC DNA FFPE Kit at 15–30°C.

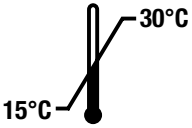
Safety Information: The cartridges contain ethanol and isopropanol. These substances should be considered flammable, harmful and irritants.

The Maxwell® CSC DNA FFPE Kit components are designed to be used with potentially infectious substances. Wear appropriate personal protective equipment (e.g., gloves and goggles) when handling potentially infectious substances. Adhere to your institutional guidelines for the handling and disposal of all potentially infectious substances used with this system.

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

Additional Information: The Maxwell® CSC DNA FFPE Kit components are qualified and quality control tested to work together. It is not recommended to mix kit components between different kit lots. Use only the components provided in the kit.

Symbols Key

Symbol	Explanation	Symbol	Explanation
	In Vitro Diagnostic Medical Device		Authorized Representative
	Store at 15–30°C.		Manufacturer
	Caution		Irritant
	Carcinogen		Contains sufficient for "n" tests
	Conformité Européenne		Warning. Biohazard.
	Warning. Pinch point hazard.		Catalog number
	Lot number		Do not reuse

3. Product Intended Use

The Maxwell® CSC DNA FFPE Kit is intended for use, in combination with the Maxwell® CSC Instruments and the Maxwell® CSC FFPE DNA purification method, as an in vitro diagnostic (IVD) medical device to perform automated isolation of DNA from FFPE (formalin-fixed, paraffin-embedded) tissue samples. The purified DNA is suitable for use in amplification-based in vitro diagnostic assays.

The Maxwell® CSC DNA FFPE Kit is intended to be used at a temperature between 15°C and 30°C. Use outside of this temperature range may result in suboptimal results.

FFPE samples prepared using 10% neutral-buffered formalin can be used with the Maxwell® CSC DNA FFPE Kit.

The Maxwell® CSC DNA FFPE Kit is not intended for use as part of a specific diagnostic test.

The Maxwell® CSC DNA FFPE Kit is intended for professional use only. Diagnostic results obtained using the DNA purified with this system must be interpreted in conjunction with other clinical or laboratory data.

4. Product Use Limitations

The Maxwell® CSC DNA FFPE Kit is only intended for use with FFPE tissue samples. It is not intended for use with non-FFPE tissue samples, such as fresh or frozen tissue samples. The Maxwell® CSC DNA FFPE Kit is not intended for use with other types of samples, including non-human samples, or for the purification of RNA.

The Maxwell® CSC DNA FFPE Kit is not intended for use with tissue samples that have been prepared with fixatives other than 10% neutral-buffered formalin.

The Maxwell® CSC DNA FFPE Kit performance has been evaluated by isolating DNA from FFPE tissue samples ranging in size from 0.1mm^3 to 2.0mm^3. It is not intended for use with samples outside of this range.

The user is responsible for establishing performance characteristics necessary for downstream diagnostic applications. Appropriate controls must be included in any downstream diagnostic applications using DNA purified using the Maxwell® CSC DNA FFPE Kit.

5. Before You Begin

Materials to be Supplied by the User

- microcentrifuge
- pipettors and pipette tips for preprocessing sample transfer into prefilled reagent cartridges
- 1.5–2.0ml tubes for incubation of samples (e.g., Microtubes, 1.5ml [Cat.# V1231])
- heating blocks set at 56°C and at 80°C
- FFPE samples with a total tissue volume of 0.1 to 2.0 mm^3 (**Note:** Samples should be stored at room temperature [15–30°C].)
- razor blades (**Note:** Use caution when using razor blades to scrape sample from the slide.)

5.A. Preparation of FFPE Samples

Preprocessing of Section Samples

1. Place section into 1.5ml microcentrifuge tube. If using slide-mounted tissue sections, scrape section off of slide using a clean razor blade.
2. Add 300µl of Mineral Oil to the sample tubes. Vortex for 10 seconds.
3. Heat the samples to 80°C for 2 minutes. Place samples at room temperature while preparing the master mix.
4. Prepare a master mix of the Lysis Buffer, Proteinase K and Blue Dye as shown below.

Reagent	Amount/Reaction	Reactions (number to be run + 1)	Total
Lysis Buffer	224µl	n + 1	224 × (n + 1)µl
Proteinase K	25µl	n + 1	25 × (n + 1)µl
Blue Dye	1µl	n + 1	1 × (n + 1)µl

5. Add 250µl of master mix to each sample tube, and vortex for 5 seconds.
6. 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 aqueous phase with a pipette.
7. Transfer the sample tubes to 56°C heat block and incubate for 30 minutes.
8. Transfer the sample tubes to 80°C heat block and incubate for 4 hours.
9. Transfer the sample tubes to the bench and allow the sample to cool to room temperature for 5 minutes.
10. Add 10µl of RNase A to the blue, aqueous phase of each sample tube. Mix by pipetting.
11. Incubate for 5 minutes at room temperature (15–30°C). During this incubation, prepare cartridges as described in Section 5.B.
12. Centrifuge at full speed in a microcentrifuge for 5 minutes.
13. Immediately transfer blue, aqueous phase containing the DNA to well #1 of the Maxwell® CSC DNA FFPE cartridge.

5.B. Maxwell® CSC DNA FFPE Cartridge Preparation

1. Change gloves before handling Maxwell® FFPE Cartridges, CSC/RSC Plungers and Elution Tubes. Cartridges are set up in the deck tray(s) outside of the instrument, and the deck tray(s) containing the cartridges and samples are transferred to the instrument for purification. Place each cartridge in the deck tray with well #1 (the largest well in the cartridge) farthest away from the Elution Tubes (Figure 2). Press down on the cartridge to snap it into position. Ensure both cartridge ends are fully seated in the deck tray. Carefully peel back the seal so that the entire seal is removed from the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed from the cartridge.

Caution: Handle cartridges with care. Seal edges may be sharp.

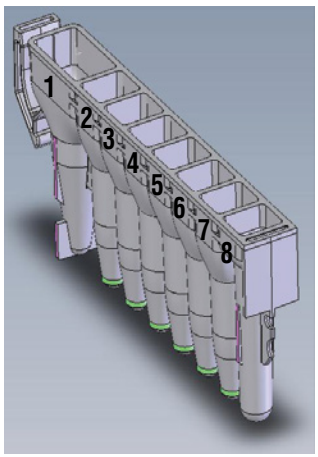
2. Place one plunger into well #8 of each cartridge.
3. Place an empty Elution Tube into the Elution Tube position for each cartridge in the deck tray(s).

   Note: Use only the elution tubes provided in the Maxwell® CSC DNA FFPE Kit. Other elution tubes may not be compatible with the Maxwell® CSC Instruments and may affect DNA purification performance.
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® CSC DNA FFPE Kit. Use of other Elution Buffers may impact DNA purification.

Maxwell® CSC DNA FFPE Cartridge Preparation Notes

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



Well Content User Adds:
1. Preprocessed sample
8. CSC/RSC Plunger

Figure 1. Maxwell® CSC Cartridge. Preprocessed FFPE sample is added to well #1, and a plunger is added to well #8.

Figure 2. Setup and configuration of the deck tray. Nuclease-Free Water is added to the Elution Tubes as indicated.

6. Instrument Run

The Maxwell® CSC DNA FFPE Method for the Maxwell® CSC Instrument can be downloaded from the Promega Web site: **www.promega.com/resources/tools/maxwellcscmethod**. The Maxwell® CSC DNA FFPE Method for the Maxwell® CSC 48 Instrument can be downloaded from the Promega web site: **www.promega.com/resources/tools/maxwellcsc48method**

1. Turn on the Maxwell® Instrument and Tablet PC. Log into the Tablet PC and start the Maxwell® IVD-mode software by double-touching the icon on the desktop. The instrument will proceed through a self-check and home all moving parts.
2. Select **Start** on the 'Home' screen.
3. Scan or enter the bar code in the upper right corner of the Maxwell® CSC DNA FFPE Kit label and press **OK** to automatically select the method to be run (Figure 3).

   Note: The Maxwell® CSC DNA FFPE Kit method bar code is required for DNA purification on the Maxwell® CSC Instruments. The kit label contains two bar codes. The method bar code is indicated in Figure 3 below. If the bar code cannot be scanned, contact Promega Technical Services.

6. Instrument Run (continued)

Figure 3. Kit label indicating the bar code to scan. The bar code to scan for starting a purification run is shown in the red box, in the upper right of the kit label.

4. On the 'Cartridge Setup' screen, touch the cartridge positions to select/deselect any positions to be used for this extraction run. Enter any required sample tracking information and press the **Proceed** button to continue.

   Note: When using the Maxwell® CSC 48 Instrument, press the **Front** or **Back** button to select or deselect cartridge positions on each deck tray.

5. After the door has opened, confirm that all extraction checklist items have been performed. Verify that preprocessed samples were added to well #1 of the cartridges, cartridges are loaded on the instrument, uncapped elution tubes are present with Elution Buffer and plungers are in well #8. Transfer the deck tray(s) containing the prepared cartridges to 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: Check the identifier on 24-position Maxwell® deck trays to determine whether they should be placed in the front or back of the instrument.

6. Confirm that all indicated preprocessing has been performed, and touch **Start** to close the instrument door and start processing.

   Note: When using a 48-position Maxwell® Instrument, if the Vision System has been enabled, the deck trays 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.

Warning: Pinch point hazard.

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

   Notes:

   1. Touching the **Abort** button will abandon the run. All samples from an aborted run will be lost.
   2. 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.

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

End of Run

9. Follow on-screen instructions at the end of the method to open 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 Operating Manual appropriate to your Maxwell® Instrument (see Table 1) to perform a **Clean Up** process to attempt to unload the plungers.

10. Cap and remove Elution Tubes containing DNA immediately following the run to prevent evaporation of the eluates. Remove the Maxwell® deck tray(s) from the instrument.

    Note: To remove the deck tray from the instrument platform, hold the deck tray by its sides. Ensure the samples are removed from the instrument before running a UV sanitization protocol to avoid damage to the purified nucleic acid. DNA samples may be stored for up to one week at +4°C and up to one month at –20°C.

11. Remove the cartridges and plungers from the deck tray(s), and discard as hazardous waste according to your institution's procedures. Cartridges, plungers and elution tubes are intended for single use. Do not reuse Maxwell® FFPE Cartridges, CSC/RSC Plungers or Elution Tubes.

7. Post-Purification

Determine that the purified DNA sample yield meets the input requirements for the appropriate downstream diagnostic 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 samples may overestimate yield; we recommend using other methods for determining yield (1).

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8. 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
Lower than expected concentration of DNA in eluate (A typical FFPE section should yield amplifiable DNA depending on tissue size, cellularity, formalin fixation condition and handling.)	Kit performance has been evaluated by isolating DNA from FFPE tissue samples ranging in size from 0.1mm^3 to 2.0mm^3. It was not designed for samples outside this range. Use sections that fall within this range.
	The kit was designed for use with FFPE tissue samples. It was not designed for use with non-FFPE tissue samples, such as fresh or frozen tissue samples. Incubation times and temperatures were tested to ensure optimal yield.
	The kit was not designed for use with tissue samples that have been prepared with fixatives other than 10% neutral-buffered formalin. Check with the pathology lab or vendor to ensure that an alternative fixative was not used.
	No claims are made for stained slides or sections. Repeat the purification with an unstained slide 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 to assess purification.
Lower than expected quality (The eluate contains highly fragmented DNA or inhibitors of downstream assays.)	The tissue section used for purification may include fragmented DNA due to formalin fixation conditions or handling. If the DNA is fragmented prior to extraction purification, fragmented DNA will be purified with this kit. Repeat with an adjacent section to assess whether there is a problem with the section or with the process.
	Some amplification-based assays are particularly sensitive to the presence of inhibitors. Downstream assay controls should identify the presence of an amplification inhibitor in the eluate. It is the user's responsibility to verify the compatibility of this product with the downstream assays.

9. Reference

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

10. Related Products

Instrument and Accessories

Product	Size	Cat.#
Maxwell® CSC Instrument*	1 each	AS6000
Maxwell® RSC/CSC Deck Tray	1 each	SP6019
Maxwell® CSC 48 Instrument*	1 each	AS8000
Maxwell® RSC/CSC 48 Front Deck Tray	1 each	AS8401
Maxwell® RSC/CSC 48 Back Deck Tray	1 each	AS8402
Microtube, 1.5ml	1,000/pack	V1231

*For In Vitro Diagnostic Use. This product is only available in certain countries.

Maxwell® CSC Reagent Kits

Visit **www.promega.com** for a list of available Maxwell® CSC purification kits.

11. Summary of Changes

The following changes were made to the 3/21 revision of this document:

1. Included use instructions for the Maxwell® CSC 48 Instrument.
2. Updated Section 6.
3. Updated Section 10.
4. Updated patent statement.
5. Updated cover page.

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