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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CE

INSTRUCTIONS FOR USE OF PRODUCT
AS1350

IVD

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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.

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

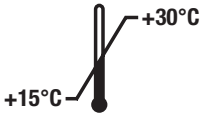
Principle of the Method: The Maxwell® CSC DNA FFPE Kit purifies nucleic acid using paramagnetic particles, which provide a mobile solid phase to optimize sample capture, washing and purification of gDNA. The Maxwell® CSC Instruments are magnetic particle-handling instruments. This system allows efficient 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.

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® CSC 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, 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°C to +30°C.

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

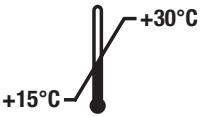
The Maxwell® CSC DNA FFPE Kit components are designed to be used with potentially infectious substances. Wear appropriate personal protective equipment (e.g., gloves and safety glasses) when handling potentially infectious substances. Adhere to your institutional guidelines for the handling and disposal 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. Do not use cartridges if the seal on the cartridge is not intact on receipt.

2. Product Components, Storage Conditions and Symbols Key (continued)

Symbols Key

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

3. Product Intended Purpose/Intended Use

The Maxwell® CSC DNA FFPE Kit is intended for use, in combination with the Maxwell® CSC Instruments and the Maxwell® CSC DNA FFPE 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 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 0.02–2.0mm^3.

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 (**Note:** Heating blocks set at 56°C and 70°C are needed if performing the optional overnight incubation.)
- FFPE samples with a total tissue volume up to 2.0mm^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 from the 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. Choose one of the following incubation times and temperatures:
   a. **Standard method:** Transfer the sample tubes to 80°C heat block and incubate for 4 hours.
   b. **Optional method:** Incubate the sample overnight at 70°C for 14–18 hours.

   Note: For lower sample input volumes (less than 0.1mm^3), the optional overnight incubation at 70°C may not be optimal. Use the standard method of 4 hours at 80°C if the overnight incubation fails to purify sufficient DNA concentration for lower input volume samples.
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.

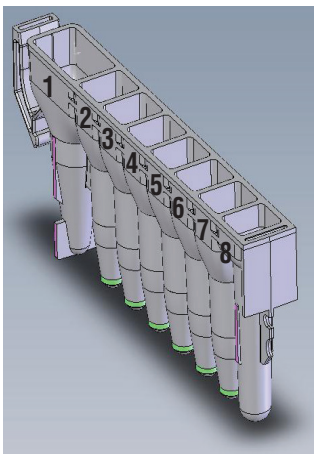
2. Place one plunger into well #8 of each cartridge.
3. Place an empty Elution Tube into the Elution Tube position for each cartridge in the deck tray(s).

   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/software-firmware/maxwell-maxprep/maxwell-cscsoftware-firmware-methods/**. The Maxwell® CSC DNA FFPE Method for the Maxwell® CSC 48 Instrument can be downloaded from the Promega web site:
www.promega.com/resources/software-firmware/maxwell-maxprep/maxwell-csc-48-methods/

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 touch **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. If the bar code cannot be scanned, contact Promega Technical Services.

Figure 3. Kit label indicating the bar code to scan. Scan the bar code shown in the red box, in the upper right of the kit label, to start a purification run.

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 touch the **Proceed** button to continue.

   Note: When using the Maxwell® CSC 48 Instrument, touch 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:

   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.

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

6. Instrument Run (continued)

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 can 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).

8. Analytical Performance Evaluation

Analytical performance of the Maxwell® CSC DNA FFPE Kit was evaluated using human FFPE tissue specimens on the Maxwell® CSC Instrument. Equivalent performance of the Maxwell® CSC DNA FFPE Kit with the Maxwell® CSC 48 Instrument was demonstrated as part of development of that instrument.

8.A. Amplifiability

Table 2. Amplifiability of DNA Purified from FFPE Tissue Sections. DNA was purified from typical-sized, single FFPE tissue sections using the Maxwell® CSC DNA FFPE Kit with standard and overnight preprocessing methods. The quantity of extracted DNA was assessed by real-time PCR targeting RNase P (102bp) as the quantification target. Amplification of the telomerase reverse transcriptase (TERT; 164bp) gene was measured as a larger sized, single-copy gene target to assess DNA quality. Some specimens that failed to amplify were traced to poor quality of the input specimens, as a similar failed result was seen when using a competitor kit to purify DNA from the specimens. Data from specimens determined to be of poor quality were excluded from analysis. The average DNA concentration for each set of replicates is shown. All FFPE tissue sections yielded at least 100 copies/µl of RNase P and were detectable for TERT when preprocessed using the standard and overnight methods.

Table 2. Amplifiability of DNA Purified from FFPE Tissue Sections (continued).

		Specimen 1		Specimen 2		Specimen 3	
		Concentration (copies/µl) or Detection		Concentration (copies/µl) or Detection		Concentration (copies/µl) or Detection	
Tissue	Preprocessing Conditions	RNase P	TERT	RNase P	TERT	RNase P	TERT
Breast*	Standard	439	Detected				
	Overnight	273	Detected				
Colon*	Standard	1313	Detected	2277	Detected		
	Overnight	983	Detected	1050	Detected		
Esophagus*	Standard	366	Detected	1314	Detected		
	Overnight	243	Detected	755	Detected		
Liver*	Standard	2472	Detected	475	Detected		
	Overnight	2206	Detected	434	Detected		
Lung	Standard	2939	Detected	3006	Detected	5217	Detected
	Overnight	1176	Detected	1510	Detected	3230	Detected
Pancreas	Standard	570	Detected	738	Detected	110	Detected
	Overnight	454	Detected	565	Detected	114	Detected
Prostate	Standard	936	Detected	1003	Detected	3064	Detected
	Overnight	829	Detected	634	Detected	1931	Detected
Stomach	Standard	659	Detected	548	Detected	404	Detected
	Overnight	454	Detected	245	Detected	223	Detected
Bladder	Standard	482	Detected	421	Detected	296	Detected
	Overnight	355	Detected	331	Detected	262	Detected
Small Intestine	Standard	741	Detected	424	Detected	1903	Detected
	Overnight	441	Detected	389	Detected	1070	Detected
Uterus	Standard	2124	Detected	3921	Detected	4081	Detected
	Overnight	1556	Detected	3117	Detected	2532	Detected

*Breast tissue from one specimen was tested. Colon, esophagus and liver tissues from two different specimens were tested. For all other tissue types, three different specimens were tested. Darkly shaded table cells indicate samples not tested.

8.A. Amplifiability (continued)

Table 3. Reproducibility of DNA Amplification. Three separate users purified DNA from FFPE tissue sections of reduced size using the Maxwell® CSC DNA FFPE Kit. DNA was purified from 0.02mm^3 and 0.1mm^3 colon and liver tissue sections following the standard preprocessing method. DNA concentration was determined by qPCR in duplicate using an RNase P target (102bp), and the average DNA concentration for each tissue section and each tissue type was calculated for each user. The lowest average DNA concentration across all users and all tissues for 0.02mm^3 and 0.1mm^3 of tissue was 45 copies/µl and 260 copies/µl, respectively.

Tissue	User ID	Tissue Sample Size	Concentration (copies/µl)
Colon	1	0.10mm^3	332
		0.02mm^3	108
	2	0.10mm^3	445
		0.02mm^3	188
	3	0.10mm^3	383
		0.02mm^3	45
Liver	1	0.10mm^3	401
		0.02mm^3	54
	2	0.10mm^3	307
		0.02mm^3	73
	3	0.10mm^3	260
		0.02mm^3	68

8.B. Reproducibility

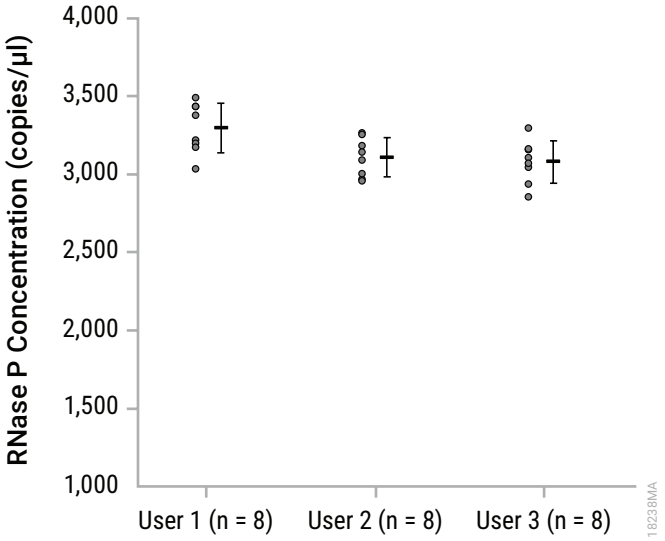


Figure 4. User-to-user reproducibility of DNA purification was characterized by three users purifying DNA from serial sections of an FFPE breast tissue specimen. Eluates were amplified by qPCR using an RNase P target (102bp) and the average DNA concentrations and inter- and intra-run CV values were calculated. Intra-run CV values were 4.9% (user 1), 4.0% (user 2) and 4.5% (user 3), and the inter-run CV across all three users was 5.3%, demonstrating reproducibility of DNA purification by each user and across multiple users. For each data set, dots on the left represent individual sample values, while the mean with standard deviation is shown on the right.

8.B. Reproducibility (continued)

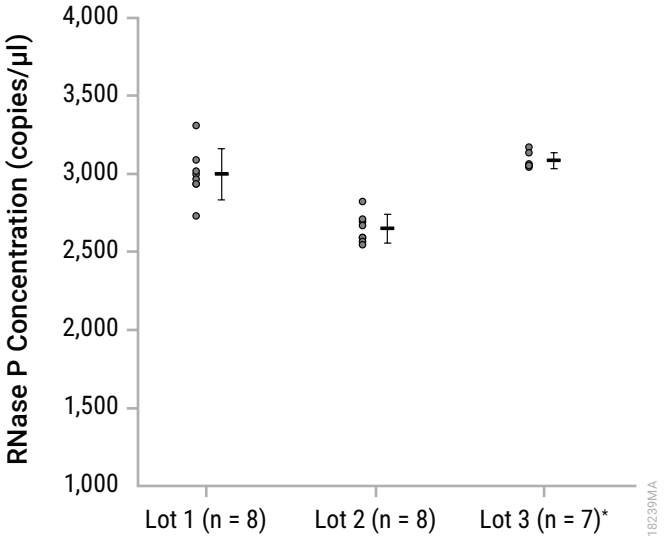


Figure 5. Lot-to-lot reproducibility of DNA purification was characterized by a single user purifying DNA from serial sections of an FFPE breast tissue specimen using three lots of the Maxwell® CSC DNA FFPE Kit. Eluates were amplified by qPCR using an RNase P target (102bp) and the average DNA concentrations were calculated, demonstrating reproducibility of DNA purification using each lot. For each data set, dots on the left represent individual sample values, while the mean with standard deviation is shown on the right. *Dixon's Outlier Test allowed exclusion of one replicate in this set as an outlier at the 95% confidence threshold. This replicate was excluded from analysis.

8.C. Inhibition (Interfering Substances)

Table 4. Analyzing Purified DNA for Interfering Substances. DNA was purified from one or two typical-sized, slide-mounted FFPE colon and liver tissue sections using standard and overnight preprocessing methods with the Maxwell® CSC DNA FFPE Kit. For each purified DNA, two sets of amplifications were assembled using 2µl and 8µl of DNA input, and the difference in C_q values (ΔC_q) between the two DNA inputs was calculated. A ΔC_q of 2 would be expected for a fourfold difference in DNA input. All conditions tested resulted in ΔC_q values of 2 ± 1 cycle, demonstrating that any potential interfering substances that copurified with the DNA did not interfere with downstream amplification.

FFPE Tissue Type	Preprocessing Conditions	Number of FFPE Tissue Sections	RNase P		
			Average 2µl C_q (Cycles)	Average 8µl C_q (Cycles)	Average ΔC_q (Cycles)
Colon	Standard	1 (n = 3)	26.4	24.5	1.9
		2 (n = 3)	25.7	23.6	2.0
	Overnight	1 (n = 2)	28.3	26.0	2.3
		2 (n = 3)	27.5	25.0	2.4
Liver	Standard	1 (n = 3)	25.5	23.5	2.0
		2 (n = 3)	24.5	22.6	1.9
	Overnight	1 (n = 3)	26.8	24.7	2.1
		2 (n = 3)	25.8	23.7	2.0

8.D. Cross Contamination

DNA was extracted from eight replicate sections of an FFPE lung tissue specimen and eight negative controls (water) using the Maxwell® CSC DNA FFPE Kit. Maxwell® CSC cartridges containing the preprocessed FFPE samples or negative control (water) were processed in alternating deck positions in the Maxwell® CSC Instrument. To determine if any cross contamination between samples had occurred, the resulting eluates were tested in duplicate by qPCR targeting the RNase P (102bp) gene to detect any DNA contamination in the negative controls from neighboring FFPE samples. No contaminating DNA was detected in any of the negative controls.

9. Clinical Performance Evaluation

Clinical performance of the Maxwell® CSC DNA FFPE Kit was evaluated by an external clinical laboratory using human FFPE tissue specimens and the Maxwell® CSC Instrument.

9.A. DNA Amplifiability

Table 5. Amplifiability of DNA Extracted from FFPE Tissues. DNA extracted from a total of 21 FFPE tissue specimens using the Maxwell® CSC DNA FFPE Kit and Maxwell® CSC Instrument was amplified in a qPCR assay targeting the wild-type c-KIT gene using the test laboratory's COLD-PCR C-KIT D816V test. DNA extracted from the same specimens using the laboratory's standard nucleic acid purification method (Laboratory Reference Method) was amplified in the same assay for comparison purposes. The difference in C_q values (ΔC_q) between DNA purified from the same FFPE tissue specimen using the Maxwell® CSC DNA FFPE Kit and the Laboratory Reference Method is shown. The amplifiability of DNA purified from the Maxwell® CSC DNA FFPE Kit correlates with the Laboratory Reference Method.

	Average C_q		
FFPE Tissue Specimen	**Maxwell® CSC DNA FFPE Kit**	**Laboratory Reference Method**	**ΔC_q**
1	26.57	27.84	–1.27
2	26.65	27.34	–0.68
3	24.16	25.23	–1.07
4	27.05	28.66	–1.62
5	24.64	25.11	–0.47
6	24.54	26.02	–1.48
7	24.25	25.00	–0.75
8	24.59	24.75	–0.16
9	25.07	25.44	–0.37
10	25.78	25.49	0.29
11	24.85	24.80	0.05
12	27.06	25.17	1.89
13	24.40	24.55	–0.15
14	23.51	24.65	–1.14
15	24.25	24.04	0.22
16	27.13	29.22	–2.09
17	27.03	28.70	–1.68
18	29.34	28.76	0.58
19	26.58	28.54	–1.96
20	26.66	27.70	–1.04
21	26.60	28.20	–1.60

9.B. Reproducibility

Table 6. Reproducibility of Results Between Testers. To demonstrate consistency of results between testers in the typical user environment, DNA extracted from seven different FFPE tissue specimens by two separate testers using the Maxwell® CSC DNA FFPE Kit was amplified by qPCR targeting the wild-type c-KIT gene using the test laboratory's COLD-PCR C-KIT D816V test. Eluates from the same FFPE tissue specimen were tested in the same assay to minimize the effect of qPCR assay variability on the results. The difference in C_q values (ΔC_q) obtained using DNA purified from the same FFPE tissue specimen by two different testers is shown in the table. The ΔC_q between testers ranged from 0.12–0.97, demonstrating reproducibility of DNA amplified by multiple testers.

	Average C_q Value		
FFPE Tissue Specimen	**Tester 1**	**Tester 2**	**ΔC_q**
1	26.57	27.54	0.97
2	26.65	26.53	0.12
3	24.16	24.39	0.23
4	27.05	26.64	0.40
5	24.64	24.29	0.36
6	24.54	24.47	0.07
7	24.25	24.06	0.20

9.C. Cross Contamination

Cross contamination occurring between samples during DNA extraction using the Maxwell® CSC DNA FFPE Kit in the typical user environment was assessed. DNA extraction using the Maxwell® CSC DNA FFPE Kit was performed with eight different FFPE tissue specimens and eight negative controls (water) in the same instrument run. Maxwell® CSC cartridges containing FFPE tissue specimens or negative controls (water) were processed in alternating, adjacent deck positions in the Maxwell® CSC Instrument. The resulting eluates were tested in duplicate by qPCR targeting the wild-type c-KIT gene using the test laboratory's COLD-PCR C-KIT D816V test to detect any contaminating DNA in the negative controls from the adjacent FFPE samples. No contaminating DNA was detected in the eight negative controls.

10. 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 up to 2.0mm^3. It was not designed for sample volumes larger than 2.0mm^3. Only use sections that meet the size specification.
	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.
	For lower sample input volumes (less than 0.1mm^3), the optional overnight incubation at 70°C may not be optimal. Use the standard decrosslinking of 4 hours at 80°C if the overnight incubation fails to purify sufficient DNA concentration for lower input volume samples.
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 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 downstream assays.

10. Troubleshooting (continued)

Any serious incident that occurred in relation to the device that led to, or might lead to, death or serious injury of a user or patient should be immediately reported to the manufacturer. Users based in the European Union should also report any serious incidents to the Competent Authority of the Member State in which the user and/or the patient is established.

11. Reference

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

12. 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.

13. Summary of Changes

The following changes were made to the 10/22 revision of this document:

1. Section 3 updated to Product Intended Purpose/Intended Use.
2. Sections 8 and 9 were added.
3. Updated document for compliance with Regulation (EU) 2017/746 on in vitro diagnostic medical devices.

[a]U.S. Pat. No. 7,329,488 and S. Korean Pat. No. 100483684.