TECHNICAL MANUAL

Maxwell® CSC Genomic DNA Kit

Instructions for Use of Product
AS1850

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

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CE

INSTRUCTIONS FOR USE OF PRODUCT
AS1850

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TM677

Maxwell® CSC Genomic DNA 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 Genomic DNA Kit is only available in certain countries.

1. Description

The Maxwell® CSC Genomic DNA Kit[a] is used with the Maxwell® Instruments specified in Table 1 to provide an easy method for efficient, automated sample preparation and purification of genomic DNA (gDNA) from a variety of human biological samples. The Maxwell® CSC Instruments are designed for use with predispensed reagent cartridges and preprogrammed purification methods, maximizing simplicity and convenience. The Maxwell® method for the CSC Genomic DNA Kit can process from one to the maximum sample number of the Maxwell® CSC Instrument in 40 minutes. The purified DNA can be used directly in a variety of downstream applications such as PCR-based assays.

Table 1. Supported Instruments.

Instrument	Cat.#	Technical Manual	Maximum Sample Number
Maxwell® CSC	AS6000	TM457	16
Maxwell® CSC 48	AS8000	TM623	48

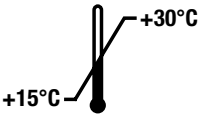
Method Principle

The Maxwell® CSC Genomic DNA Kit purifies nucleic acid from samples using paramagnetic particles, which provide a mobile solid phase to optimize sample capture, washing and purification of gDNA. Maxwell® Instruments are magnetic particle-handling instruments that efficiently bind nucleic acids to the paramagnetic particles in the first well of a prefilled cartridge. The samples are processed through a series of washes before the gDNA is eluted. This magnetic capture approach avoids common problems such as clogged tips or partial reagent transfers that result in suboptimal purification processing by other commonly used automated systems.

2. Product Components, Storage Conditions and Symbols Key

PRODUCT	SIZE	CAT.#
Maxwell® CSC Genomic DNA Kit	**48 preps**	**AS1850**

For In Vitro Diagnostic Use. Professional use only. Contains sufficient reagents for 48 automated isolations from a variety of human biological samples. Cartridges are for single use only.



Includes:

- 2 × 1ml Proteinase K (PK) Solution
- 1ml RNase A Solution
- 20ml Lysis Buffer
- 20ml Lytic Enhancer (LE2)
- 48 Maxwell® CSC Cartridges (CSCQ)
- 50 CSC/RSC Plungers
- 50 Elution Tubes (0.5ml)
- 20ml Elution Buffer

Storage Conditions: Store the Maxwell® CSC Genomic DNA Kit at +15°C to +30°C.

Safety Information: The Maxwell® CSC Cartridges (CSCQ) contain ethanol and isopropanol. These substances should be considered flammable, harmful and irritants. Refer to the Safety Data Sheet (SDS) for detailed safety information. Adhere to institutional guidelines for the handling and disposal of all chemical waste used with this system.

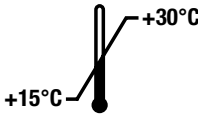
The Maxwell® CSC Cartridges (CSCQ) are designed to be used with potentially infectious substances. Wear appropriate protection (e.g., gloves and safety glasses) when handling infectious substances. Adhere to your institutional guidelines for the handling and disposal of all infectious substances when used with this system.

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

Additional Information: The Maxwell® CSC Genomic DNA Kit components are qualified and quality-control tested to work together. Do not mix kit components between different kit lots. Use only the components provided in the kit. For additional safety information, see the Safety Data Sheet, available at: **www.promega.com**.

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
	Health hazard		Corrosive
	Irritant		Flammable
	Conformité Européenne		Contains sufficient for “n” tests
	Warning. Pinch point hazard.		Caution
	Lot number		Warning. Biohazard.
	Do not reuse		Catalog number

3. Product Intended Purpose/Intended Use

The Maxwell® CSC Genomic DNA Kit is intended for use, in combination with the Maxwell® CSC Instruments and the Maxwell® CSC and Maxwell® CSC 48 Genomic DNA purification methods, as an in vitro diagnostic (IVD) medical device to perform automated isolation of genomic DNA from a variety of human biological samples. The purified genomic DNA is suitable for use in amplification-based in vitro diagnostic assays.

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

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

4. Product Use Limitations

The Maxwell® CSC Genomic DNA Kit has been validated with human whole blood, buffy coat, bone marrow, buccal swabs, tissues and cell samples. The user is responsible for validating its use to extract DNA from other sample types.

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

5. Before You Begin

Materials to Be Supplied by the User

- benchtop vortex mixer
- pipettors and pipette tips for sample transfer into prefilled reagent cartridges
- 1.5–2.0ml tubes for sample incubation are recommended (e.g., Microtubes, 1.5ml [Cat.# V1231]) Other tube types should be evaluated by the laboratory.
- dry heat block, water bath or thermal mixer set at 56°C
- deionized or Nuclease-Free Water (Cat.# MC1191) for cell pellet (Section 5.C) and tissue samples (Section 5.E)
- 1X Phosphate Buffered Saline (PBS) for cell pellet samples prepared from urine (Section 5.C)
- **optional:** Clearing Columns (Cat.# Z3871) for buccal swab samples (Section 5.F)
- **optional:** rotating tube mixer

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5.A. Preparing Whole Blood and Buffy Coat Samples

Sample Processing Capacity

The total genomic DNA yield from whole blood and buffy coat samples depends on the sample volume and number of white blood cells (WBC) per milliter. For these sample types, a sample volume range of 50–300µl can be used. During development, whole blood and buffy coat prepared from whole blood with a range of 4×10^6 to 10×10^6 WBC/ml were tested and found to provide acceptable performance. Samples outside of this range may be compatible with the extraction chemistry but should be evaluated by the laboratory for extraction performance and compatibility with downstream assays.

An elution volume range of 50–200µl can be used for whole blood samples. Because buffy coat samples generally yield a high amount of genomic DNA, we recommend eluting with 200µl to provide the most effective elution. Elution volumes of 50–200µl can be used with buffy coat samples but volumes less than 200µl may not provide optimal results.

Notes:

a. This kit has been tested with human whole blood and buffy coat samples prepared from human whole blood collected in EDTA, citrate or heparin tubes. Performance of this kit with other types of blood collection tubes should be evaluated by the user.

b. This kit has been tested with blood and buffy coat samples stored under the following conditions: stored at 15–30°C for up to 72 hours, stored at 2–10°C for up to 7 days or stored at –65°C or lower prior to DNA purification. Other sample storage conditions may provide acceptable performance but should be evaluated by the laboratory. Frozen samples should be thawed completely before processing. All blood and buffy coat samples should be thoroughly mixed before use.

1. Mix all blood or buffy coat samples for at least 5 minutes at 15–30°C. This can be accomplished by using a rotating tube mixer or intermittent mixing with a vortex mixer.
2. Proceed to Section 5.D for lysate preparation instructions.

5.B. Preparing Bone Marrow Aspirate Samples

Sample Processing Capacity

The total genomic DNA yield from bone marrow aspirate samples depends on the total number of cells being processed. During development, bone marrow aspirate samples in the volume range of 50–300µl were tested and found to provide acceptable performance. Samples outside of this range may be compatible with the extraction chemistry but should be evaluated by the laboratory for extraction performance and compatibility with downstream assays.

Because bone marrow aspirate samples generally yield a high amount of genomic DNA, we recommend eluting with 200µl to provide the most effective elution. Elution volumes of 50–200µl can be used with bone marrow samples but volumes less than 200µl may not provide optimal results.

Notes:

a. This kit has been tested with human bone marrow aspirate samples collected in EDTA, citrate or heparin tubes. Performance of this kit with other types of blood collection tubes should be evaluated by the user.

b. This kit has been tested with bone marrow aspirate samples stored frozen (stored at –65°C or lower) prior to DNA purification. Other sample storage conditions may provide acceptable performance but should be evaluated by the laboratory. Frozen samples should be thawed completely before processing. All bone marrow aspirate samples should be thoroughly mixed before use.

1. Mix all bone marrow aspirate samples for at least 30 minutes at 15–30°C using a rotating tube mixer or intermittently mixing with a vortex mixer.
2. Proceed to Section 5.D for lysate preparation instructions.

5.C. Preparing Cell Pellet Samples

Sample Processing Capacity

Cell pellets can be generated from a number of sample types including biological fluids (e.g., urine or amniotic fluid), purified cells (e.g., peripheral blood mononuclear cells) or cultured cells. Centrifugation of the sample is used to generate a cell pellet and that pellet is resuspended in 300µl of nuclease-free water. The total genomic DNA yield from cell pellet samples depends on the number of cells present in the sample. During development, cell pellets from up to 5×10^6 cells were tested (see Table 2) and found to provide acceptable performance. Samples outside of this range may be compatible with the extraction chemistry but should be evaluated by the laboratory for extraction performance and compatibility with downstream assays.

Table 2. The Cell Pellet Sample Types Evaluated.

Sample Type	Sample Range Tested	Suggested Elution Volume
Urine	15–50ml	50µl
Amniotic fluid	1–5ml	50µl
Peripheral blood mononuclear cells (PBMCs)	5×10^4–5×10^6 cells	50–200µl
Cultured cells	5×10^2–5×10^6 cells	50–200µl

For cell pellet samples, use an elution volume range of 50–200µl. When processing samples that generate low cell numbers in the cell pellet, we recommend using a 50µl elution volume. For samples with more cells, a larger elution volume may result in higher genomic DNA yields. Laboratories should confirm that the elution volume for a given cell pellet sample type provides sufficient purity and concentration for their downstream assay.

Notes:

a. This kit has been tested with cell pellet samples processed immediately after generating a cell pellet and stored frozen (stored at –65°C or lower) prior to DNA purification. Other sample storage conditions may provide acceptable performance but should be evaluated by the laboratory. Frozen samples should be thawed completely before processing.

b. If sample freezing is desired, samples should be stored frozen after generating the cell pellet. Collecting a cell pellet from a sample that has been frozen and thawed can result in loss of performance.

1. Centrifuge the desired sample volume at a speed of at least 2,000 × *g* for 20 minutes to generate a cell pellet.
   a. For urine samples, wash the cell pellet by resuspending in 750µl of 1X PBS.
   b. Centrifuge the PBS-suspended sample to generate a cell pellet.
2. Decant or aspirate the liquid from the pelleted cells. Resuspend the pellet in 300µl of nuclease-free water.
3. Proceed to Section 5.D for lysate preparation instructions.

5.D. Preparing Lysates from Whole Blood, Buffy Coat, Bone Marrow and Cell Pellet Samples

1. Prepare and label incubation tubes that will fit in a heat block set at 56°C.
2. Add 30µl of Proteinase K (PK) Solution to each incubation tube.
3. Transfer the desired sample volume to each incubation tube. Change pipette tips between each sample transfer to prevent cross-contamination.

   Note: Transferring clotty, fatty or other solid material into the incubation tube can result in poor sample lysis. Only transfer liquid sample to the incubation tube.
4. Cap and vortex each tube at maximum speed for 10 seconds.
5. Add 300µl of Lytic Enhancer (LE2) to each incubation tube. Change pipette tips each time the Lytic Enhancer (LE2) is dispensed to prevent cross-contamination.

   Note: Proceed to Step 6 without mixing or vortexing.
6. Add 300µl of Lysis Buffer to each incubation tube. Change pipette tips each time the Lysis Buffer is dispensed to prevent cross-contamination.
7. Cap and vortex each tube at maximum speed for 10 seconds.

   Note: Confirm vortexing resulted in a homogeneous lysate.
8. Incubate each tube in the 56°C heat block for 20 minutes. During this incubation, prepare the Maxwell® CSC cartridges as described in Section 5.G.
9. Vortex each tube at maximum speed for 10 seconds.
10. Transfer each lysate sample from the incubation tube to well #1 of a separate cartridge and mix well with the binding solution in well #1 by aspirating and dispensing 5–10 times after transfer to make a homogeneous mixture (well #1 is the largest well in the cartridge). Change pipette tips between each sample transfer to prevent sample cross-contamination.

    Note: Failure to create a homogeneous mixture of sample lysate and binding solution in well #1 of the cartridge may result in decreased yield and purity of the final eluate.

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5.E. Preparing Lysates from Tissue Samples

Sample Processing Capacity

The total genomic DNA yield from tissue samples depends on the mass and type of tissue processed. For tissue samples, a sample range of 5–50mg can be used. During development, heart, pancreas, brain and breast tissue samples were evaluated as exemplars and found to provide acceptable performance. A wider range of tissue types may be compatible with the extraction chemistry but should be evaluated by the laboratory for extraction performance and compatibility with downstream assays.

An elution volume range of 50–200µl can be used for tissue samples. The volume of elution buffer to use will depend on the mass and type of tissue being processed. Laboratories should evaluate elution volumes that provide acceptable performance in their downstream assays for the tissue mass and types being processed.

Note: This kit has been tested with tissue samples stored frozen (stored at –65°C or lower) prior to DNA purification. Other sample storage conditions may provide acceptable performance but should be evaluated by the laboratory. Frozen samples should be thawed completely before processing.

1. To lyse tissue samples, set the temperature of a dry heat block, water bath or thermal mixer to 56°C. Prepare and label incubation tubes that will fit in the desired heating option.
2. Transfer 5–50mg of tissue to each tube. Cutting tissue into smaller fragments may decrease lysis time. Centrifuge the tube at top speed for 15 seconds to collect the tissue pieces at the bottom of the tube.
3. Add 300µl of Nuclease-Free Water (Cat.# MC1191 or equivalent) to each incubation tube.
4. Add 30µl of Proteinase K (PK) Solution to each incubation tube. Change pipette tips each time the Proteinase K (PK) Solution is dispensed to prevent cross-contamination.
5. Cap and vortex each tube at maximum speed for 10 seconds.
6. Add 300µl of Lytic Enhancer (LE2) to each incubation tube. Change pipette tips each time Lytic Enhancer (LE2) is dispensed to prevent cross-contamination.
7. Cap and vortex each tube at maximum speed for 10 seconds.
8. Incubate each tube at 56°C using one of the following options:
   a. With a thermal mixer, use a high shake speed (e.g., 1,500rpm) for up to 2 hours.
   b. With a dry heat block or water bath heater, use without shaking for at least 16 hours.
9. Vortex each tube at maximum speed for 10 seconds.
10. Centrifuge each tube at maximum speed in a microcentrifuge for 5 minutes to pellet any undigested material.
11. Transfer all of the supernatant from each incubation tube into a new tube. Avoid transferring any pelleted material. If a distinct fatty layer appears on top of the sample after centrifugation, do not transfer that layer to the new tube.
12. Add 300µl of Lysis Buffer to each new tube. Change pipette tips each time the Lysis Buffer is dispensed to prevent cross-contamination.
13. Cap and vortex each tube at maximum speed for 10 seconds.

14. Prepare cartridges as described in Section 5.G.

15. Transfer the tissue lysate sample from each tube to well #1 of a separate cartridge and mix well with the binding solution in well #1 by aspirating and dispensing at least 10 times after transfer to make a homogeneous mixture (well #1 is the largest well in the cartridge). Change pipette tips between each sample transfer to prevent sample cross-contamination.

    Notes:

    a. Transferring the tissue pellet or fatty layer from the incubation tube into the new tube may result in poor yield or purities.

    b. Failure to create a homogeneous mixture of sample lysate and binding solution in well #1 of the cartridge can result in decreased yield and purity in the final eluate.

5.F. Preparing Lysates from Buccal Swab Samples

Sample Processing Capacity

The total genomic DNA yield from buccal swab samples depends on how well buccal cells are transferred to the swab. During development, 1 and 2 buccal swabs were tested and provided acceptable performance. An elution volume range of 50–200µl can be used for buccal swab samples. Laboratories should choose an elution volume for buccal swab samples that provides sufficient purity and concentration for their downstream assay.

Note: This kit has been tested with dry buccal swab samples stored at 15–30°C prior to DNA purification. Other sample storage conditions may provide acceptable performance but should be evaluated by the laboratory.

1. Prepare and label 1.5–2.0ml incubation tubes that will fit in a heat block set at 56°C.

2. **Optional:** Place a clearing column (Cat.# Z3871) in each incubation tube.

3. Place 1–2 buccal swab head(s) in each incubation tube or clearing column in each incubation tube. Remove the stick from the buccal swab head(s) by cutting or breaking the swab stick off above the swab head so that the cap can be closed on the tube or clearing column containing the swab head.

4. In a separate tube, combine 300µl of Lytic Enhancer (LE2) with 30µl of Proteinase K (PK) Solution for each sample plus one extra sample. See the table below. For example, to process 16 samples, create a master mix for 17 reactions by combining 300µl × 17 = 5,100µl Lytic Enhancer (LE2) and 30µl × 17 = 510µl Proteinase K.

Reagent	Amount Per Reaction	Reactions (Sample Number + 1)	Total
Lytic Enhancer (LE2)	300µl	n+1	300 × (n+1)µl
Proteinase K (PK) Solution	30µl	n+1	30 × (n+1)µl

5. Mix the Lytic Enhancer (LE2)/Proteinase K (PK) Solution by inverting the tube at least 10 times.

6. Add 330µl of Lytic Enhancer (LE2)/Proteinase K (PK) Solution to each sample, and close tube. Change pipette tips each time Lytic Enhancer (LE2)/Proteinase K (PK) Solution is dispensed to prevent cross-contamination.

7. Incubate each tube at 56°C for 20 minutes. During this incubation, prepare cartridges as described in Section 5.G.

5.F. Preparing Lysates from Buccal Swab Samples (continued)

8. Use one of the following options to remove the swab head(s) from the tube:
   a. If using a Clearing Column, place the tube in a microcentrifuge and centrifuge at maximum speed for 2 minutes. Remove the tube from the microcentrifuge. Open the tube; remove and discard the clearing column containing the swab head(s).
   b. If not using a Clearing Column, use tweezers to remove the swab head(s) from the tube, carefully squeezing the remaining lysate from the swab head(s). Discard the swab head(s). Clean the tweezers and change gloves between each swab head removal to prevent cross-contamination.
9. Add 300µl of Lysis Buffer to well #1 of each cartridge to be used (well #1 is the largest well in the cartridge).
10. Transfer each swab lysate sample from the incubation tube to well #1 of a separate cartridge and mix with the Lysis Buffer and binding solution in well #1 by aspirating and dispensing 5–10 times after transfer to make a homogeneous mixture (well #1 is the largest well in the cartridge). Change pipette tips between each sample transfer to prevent sample cross-contamination.

    Note: Failure to create a homogeneous mixture of sample lysate, Lysis Buffer and binding solution in well #1 can result in decreased yield and purity in the final eluate.

5.G. Preparing the Maxwell® CSC Genomic DNA Cartridge

1. Change gloves before handling cartridges, CSC/RSC Plungers and Elution Tubes (0.5ml). Cartridges are set up in the deck tray(s) outside of the instrument before transferring the deck tray(s) containing the cartridges and samples to the instrument for purification. Place each cartridge in the deck tray(s) 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.

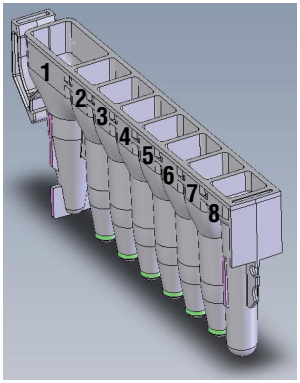
2. Add 15µl of RNase A Solution into well #3 of the Maxwell® CSC Cartridge (CSCQ).
3. Place one plunger into well #8 of each cartridge.
4. Place an empty elution tube into the elution tube position for each cartridge in the deck tray(s).

   Note: Use only the elution tubes provided in the Maxwell® CSC Genomic DNA Kit. Other elution tubes may be incompatible with the Maxwell® CSC Instruments and affect DNA purification performance.
5. Add 50–200µl of Elution Buffer to the bottom of each elution tube.

   Note: Only use the Elution Buffer provided in the Maxwell® CSC Genomic DNA Kit. Use of other elution buffers may affect DNA purification performance.
6. Proceed to Section 6, Maxwell® Instrument Run.

Maxwell® CSC Genomic DNA 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.



User Adds to Wells
1. Lysed sample
3. 15µl of RNase A Solution
8. CSC/RSC Plunger

Figure 1. Maxwell® CSC Cartridge. Lysed sample is added to well #1, 15µl of RNase A Solution is added to well #3, and a plunger is added to well #8.

Figure 2. Setup and configuration of the deck tray(s). Elution Buffer is added to the elution tubes as indicated. Deck tray shown is from the Maxwell® CSC Instrument (Cat.# AS6000).

6. Maxwell® Instrument Run

For detailed information, refer to the Technical Manual specific to your Maxwell® CSC Instrument. See Table 1.

1. Turn on the Maxwell® Instrument and Tablet PC. Log in to 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. Touch **Start** on the 'Home' screen.
3. Scan or enter the bar code in the upper right corner of the Maxwell® CSC Genomic DNA Kit label and press **OK** to automatically select the method to be run (Figure 3).

   Note: The Maxwell® CSC Genomic DNA 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. 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, confirm that the Maxwell® CSC Genomic DNA method is displayed at the top of the screen. Touch the cartridge positions to select or 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 for the appropriate deck tray.

5. After the door has been opened, confirm that all Extraction Checklist items have been performed. Verify that 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 onto the Maxwell® Instrument platform.

   Inserting the Maxwell® deck tray(s): 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. 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 trays will be scanned as the platform 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.

7. The Maxwell® Instrument will immediately begin the purification run. The screen will display the steps 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.

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 the door. Verify that plungers are located in well #8 of the cartridge at the end of the run. If plungers have not been removed from the plunger bar, follow the instructions in the Technical Manual appropriate to your Maxwell® Instrument (see Table 1) to perform a **Clean Up** process to attempt to unload the plungers.

6. Maxwell® Instrument Run (continued)

10. Remove the deck tray(s) from the instrument immediately following the run to prevent evaporation of the eluates. Remove elution tubes containing DNA, and cap the tubes.

    Note: Following the automated purification procedure, the deck tray(s) will be warm. To remove a deck tray from the instrument platform, hold onto the deck tray by its sides.

    Ensure samples are removed from the instrument before running a UV sanitation protocol to avoid damage to the nucleic acid.

11. Remove the cartridges and plungers from the Maxwell® deck tray(s). Discard as hazardous waste according to your institution's procedures. Do not reuse Maxwell® CSC Cartridges, CSC/RSC Plungers or Elution Tubes.

7. Post-Purification

Determine that the purified DNA sample yield and purity meets the input requirements for the appropriate downstream diagnostic assay prior to use in that assay.

8. Analytical Performance Evaluation

The analytical performance evaluation was performed using human samples with the Maxwell® CSC Genomic DNA Kit and the Maxwell® CSC Instruments.

8.A. DNA Yield

DNA yield was assessed using DNA purified with the Maxwell® CSC Genomic DNA Kit from fresh and frozen whole blood collected in EDTA tubes; frozen whole blood collected in citrate and heparin tubes; fresh and frozen buffy coat samples generated from whole blood collected in EDTA tubes; frozen buffy coat samples generated from whole blood collected in citrate and heparin tubes; one and two buccal swabs preprocessed with a clearing column; heart, pancreas and brain tissues; tissue culture cells; and frozen bone marrow aspirates collected in EDTA, citrate and heparin tubes.

The graphs and table in this section represent the absorbance yield of each replicate that was purified for each sample type. Each dot in the graphs represents an individual measurement on the left while the mean with standard deviation is on the right. Each data set includes a total of 12 replicates, four replicates purified using the Maxwell® CSC Instrument and eight replicates purified using the Maxwell® CSC 48 Instrument.

The tables below the figure legends describe the sample information for each sample set shown in the associated graphs.

Whole Blood

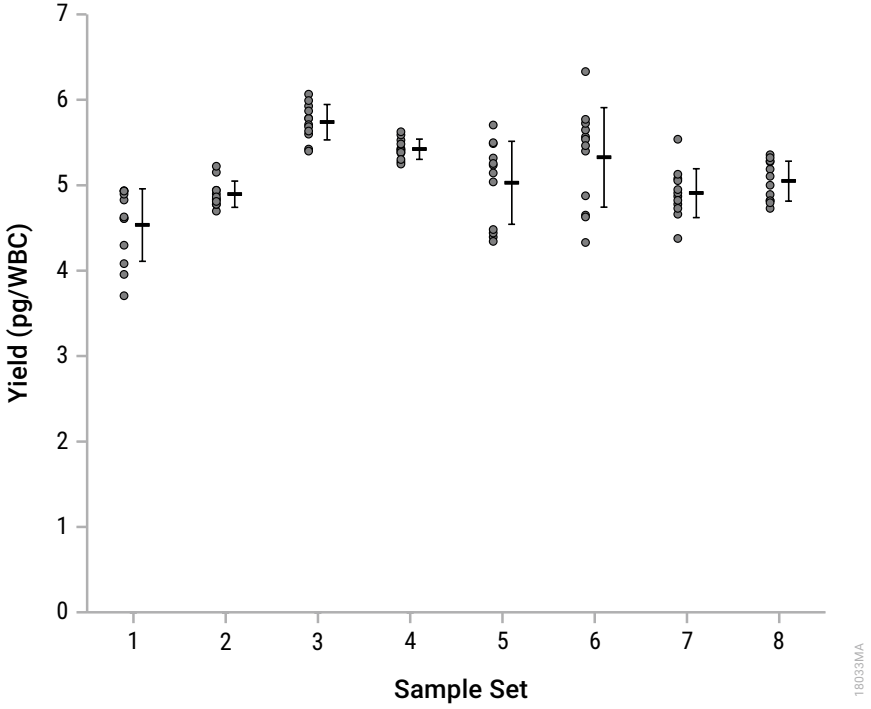


Figure 4. Whole blood DNA yield. For 300µl of fresh and frozen whole blood samples collected in EDTA tubes and frozen whole blood samples collected in citrate and heparin tubes, the average DNA yields were in the range of 4.5–5.7pg/WBC.

Sample Set	Anticoagulant	Storage	Input Volume (µl)	Elution Volume (µl)
1	EDTA	Frozen	300	50
2	EDTA	Frozen	300	200
3	EDTA	Fresh	300	50
4	EDTA	Fresh	300	200
5	Citrate	Frozen	300	50
6	Citrate	Frozen	300	200
7	Heparin	Frozen	300	50
8	Heparin	Frozen	300	200

8.A. DNA Yield (continued)

Buffy Coat

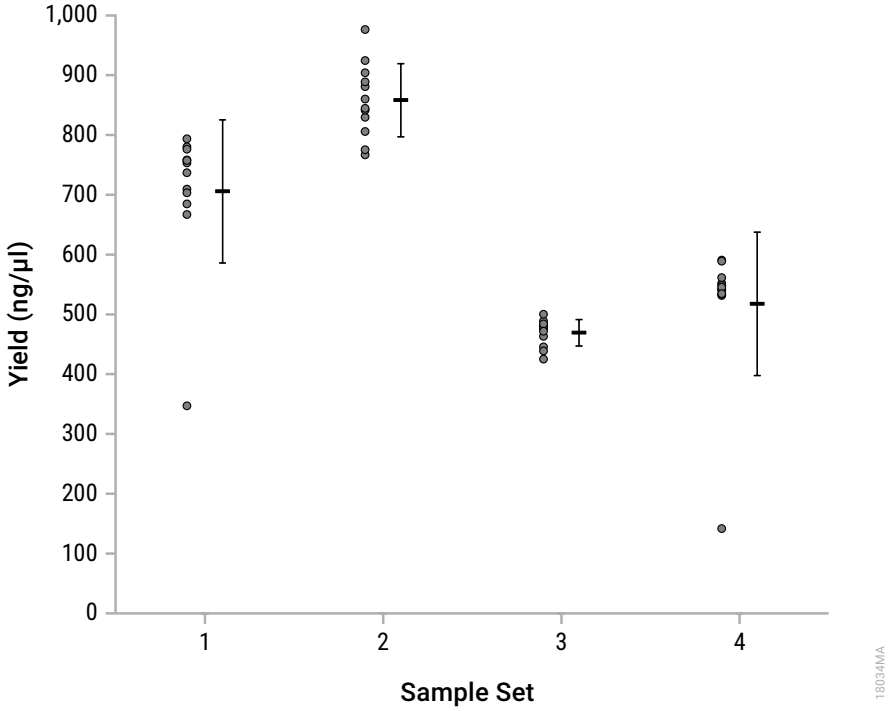


Figure 5. Buffy coat DNA yield. With a 300µl sample input volume and 200µl elution volume for fresh and frozen buffy coat samples generated from whole blood collected in EDTA tubes and frozen buffy coat samples generated from whole blood collected in citrate and heparin tubes, the average DNA concentrations were in the range of 469.3–858.2ng/µl.

Sample Set	Anticoagulant	Storage	Input Volume (µl)	Elution Volume (µl)
1	EDTA	Frozen	300	200
2	EDTA	Fresh	300	200
3	Citrate	Frozen	300	200
4	Heparin	Frozen	300	200

Buccal Swab

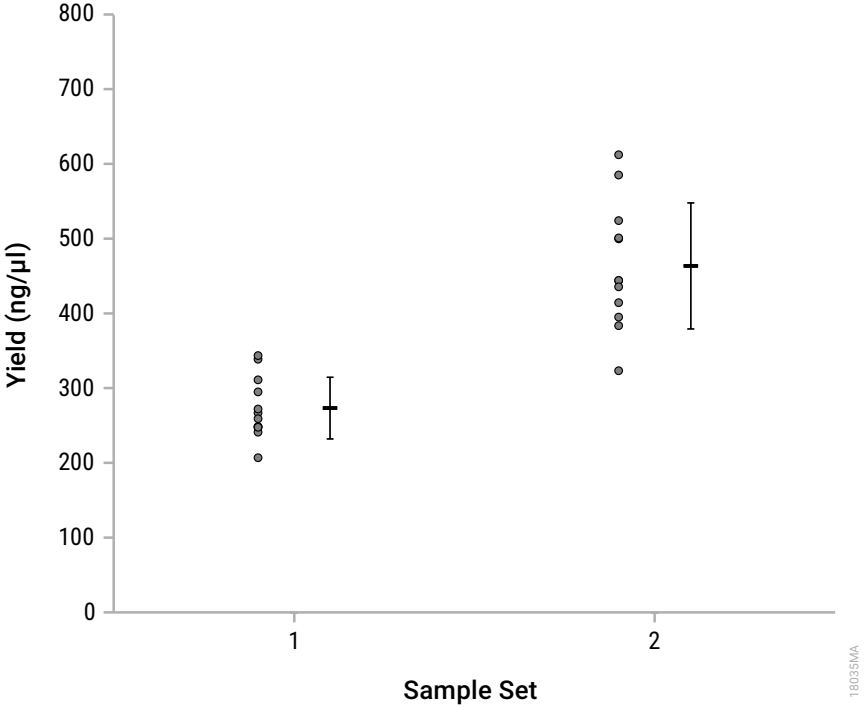


Figure 6. Buccal swab DNA yield. For one and two input buccal swabs preprocessed with a clearing column, the average DNA concentrations were in the range of 273.2–463.5ng/µl. Sample set 1 refers to one swab and sample set 2 refers to two swabs. An elution volume of 50µl was used for all samples.

8.A. DNA Yield (continued)

Tissue

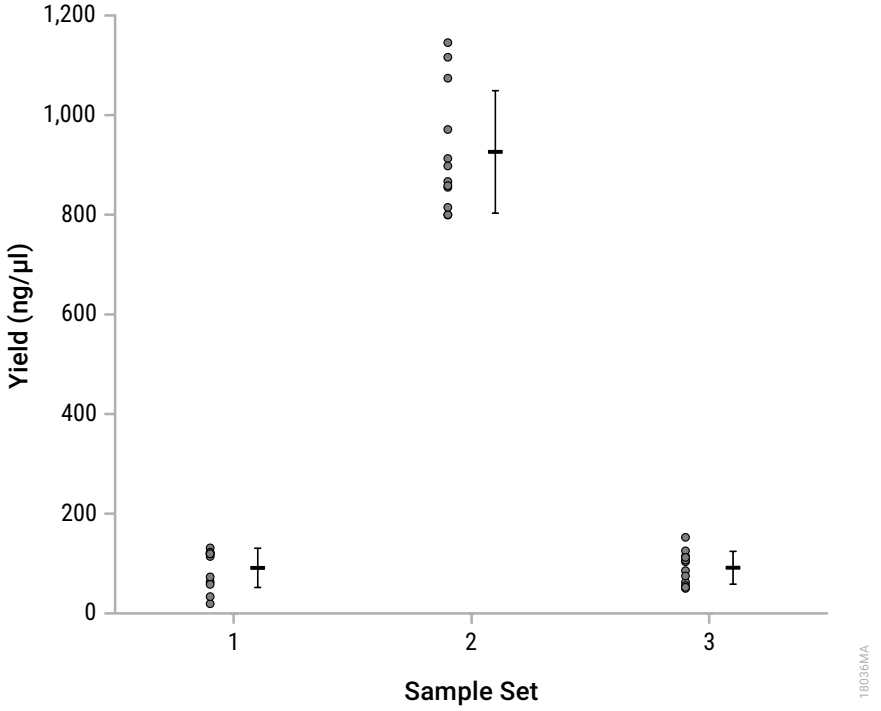


Figure 7. Tissue DNA yield. For 50mg heart, pancreas, and brain tissues with a 200µl elution volume, the average DNA concentrations were in the range of 91.2–926.0ng/µl. Sample set 1 refers to heart tissue, sample set 2 refers to pancreas tissue, and sample set 3 refers to brain tissue.

Cells

For 5×10^6 HEK293 tissue culture cells with a 200µl elution volume, the average DNA concentration was 550.2ng/µl.

Cell Type	Input Cell Number	Elution Volume	Concentration (ng/µl)
HEK293 tissue culture cells	5×10^6	200µl	523.4
			526.8
			536.1
			650.1
			481.6
			522.9
			530.4
			618.9
			546.5
			550.1
			569.9
			545.4
		Average	550.2

8.A. DNA Yield (continued)

Bone Marrow

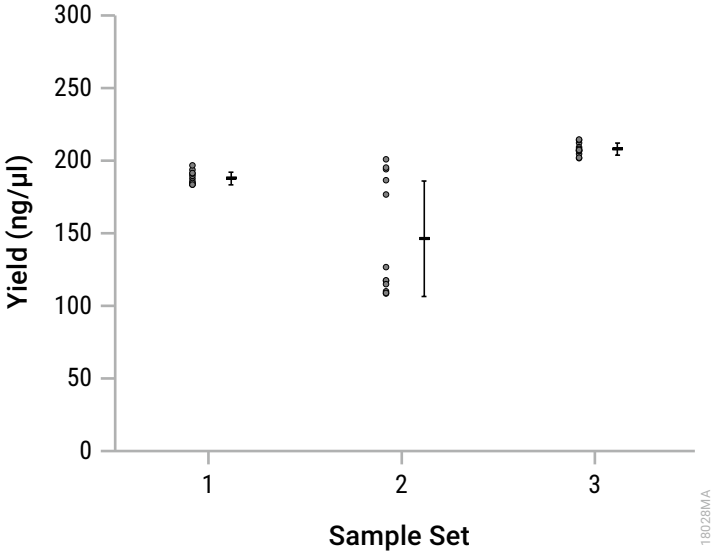


Figure 8. Bone marrow DNA yield. For 300µl of frozen bone marrow aspirates collected in EDTA, citrate and heparin tubes and with a 200µl elution volume, the average DNA concentrations were in the range of 146.3–207.8ng/µl. Sample set 1 refers to bone marrow aspirates collected in EDTA tubes, sample set 2 refers to bone marrow aspirates collected in citrate tubes, and sample set 3 refers to bone marrow aspirates collected in heparin tubes.

8.B. DNA Quality (Purity)

DNA purities were assessed using DNA purified with the Maxwell® CSC Genomic DNA Kit from fresh and frozen whole blood collected in EDTA tubes, frozen whole blood collected in citrate and heparin tubes, fresh and frozen buffy coat samples generated from whole blood collected in EDTA tubes, frozen buffy coat samples generated from whole blood collected in citrate and heparin tubes, one and two buccal swabs preprocessed with a clearing column, heart, pancreas, and brain tissues, tissue culture cells, and frozen bone marrow aspirates collected in EDTA, citrate and heparin tubes.

The graphs and table in this section represent the absorbance A_{260}/A_{280} and A_{260}/A_{230} purity ratios of each replicate that was purified for each sample type. Each dot in the graphs represents an individual measurement on the left while the mean with standard deviation is on the right. Each data set includes a total of 12 replicates, four replicates purified using the Maxwell® CSC Instrument and eight replicates purified using the Maxwell® CSC 48 Instrument.

The tables below the figure legends describe the sample information for each sample set shown in the associated graphs.

Whole Blood

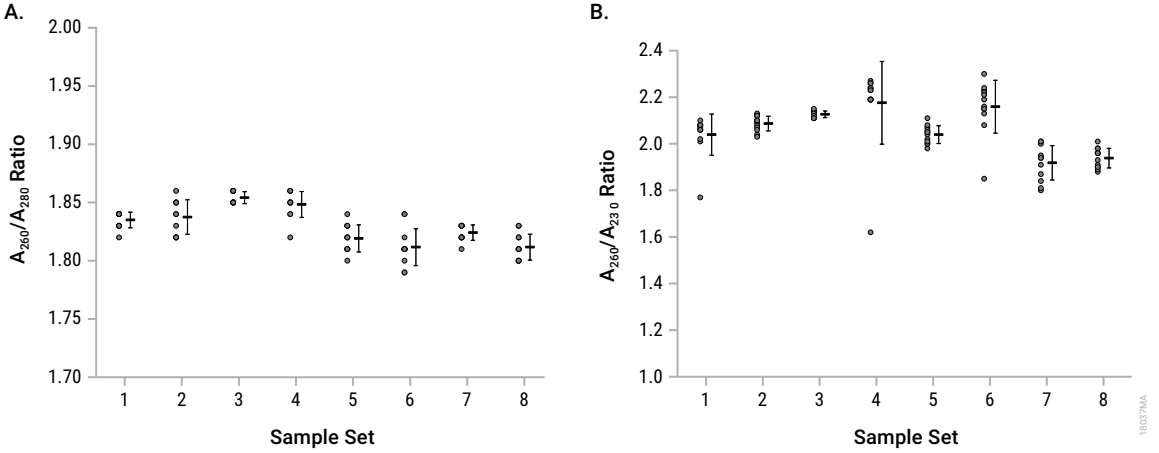


Figure 9. Whole blood DNA quality. For 300µl fresh and frozen whole blood samples collected in EDTA tubes and frozen whole blood samples collected in citrate and heparin tubes, the average A_{260}/A_{280} ratios were in the range of 1.8–1.9 (**Panel A**) and the average A_{260}/A_{230} ratios were in the range of 1.9–2.2 (**Panel B**).

Sample Set	Anticoagulant	Storage	Input Volume (µl)	Elution Volume (µl)
1	EDTA	Frozen	300	50
2	EDTA	Frozen	300	200
3	EDTA	Fresh	300	50
4	EDTA	Fresh	300	200
5	Citrate	Frozen	300	50
6	Citrate	Frozen	300	200
7	Heparin	Frozen	300	50
8	Heparin	Frozen	300	200

8.B. DNA Quality (Purity; continued)

Buffy Coat

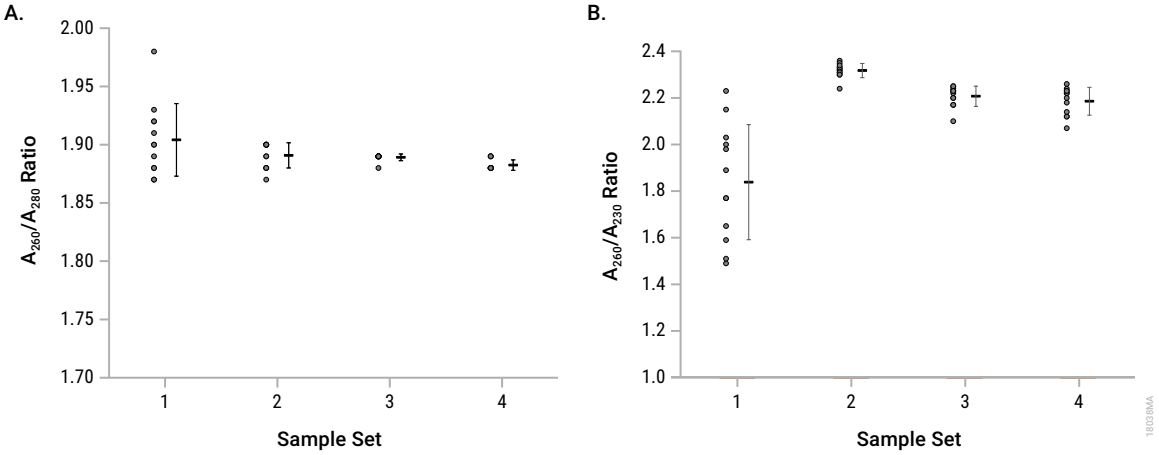


Figure 10. Buffy coat DNA quality. With 300µl sample input volume and 200µl elution volume for fresh and frozen buffy coat samples generated from whole blood collected in EDTA tubes, and frozen buffy coat samples generated from whole blood collected in citrate and heparin tubes, the average A_{260}/A_{280} ratios were around 1.9 (**Panel A**) and the average A_{260}/A_{230} ratios were in the range of 1.8–2.3 (**Panel B**).

Sample Set	Anticoagulant	Storage	Input Volume (µl)	Elution Volume (µl)
1	EDTA	Frozen	300	200
2	EDTA	Fresh	300	200
3	Citrate	Frozen	300	200
4	Heparin	Frozen	300	200

Buccal Swab

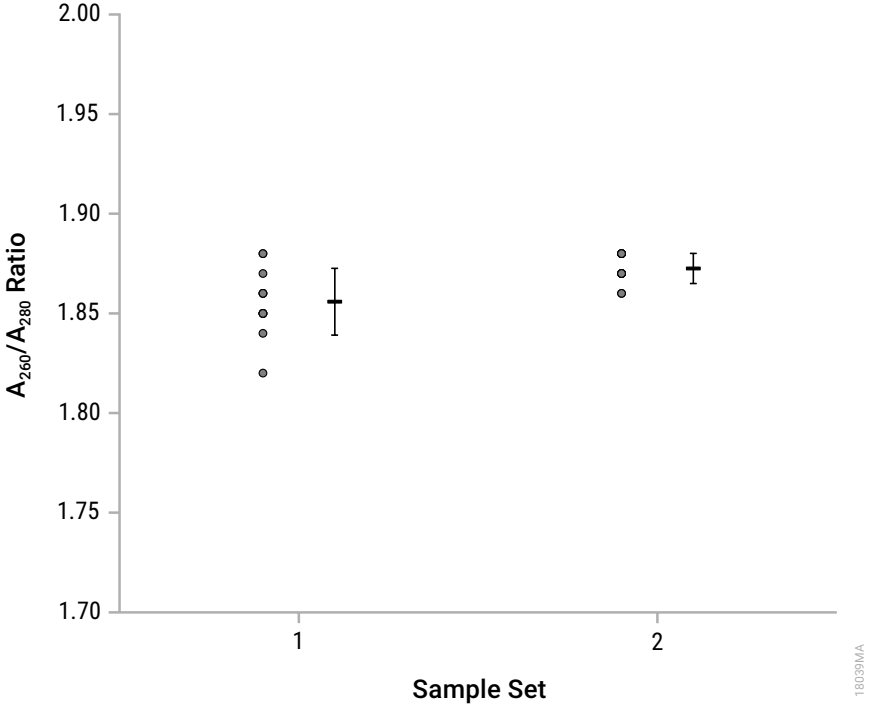


Figure 11. Buccal swab DNA quality. For one and two input buccal swabs preprocessed with a clearing column and with an 50µl elution volume, the average A_{260}/A_{280} ratios were in the range of 1.8–1.9. In the graph, sample set 1 refers to one swab and sample set 2 refers to two swabs.

8.B. DNA Quality (Purity; continued)

Tissue

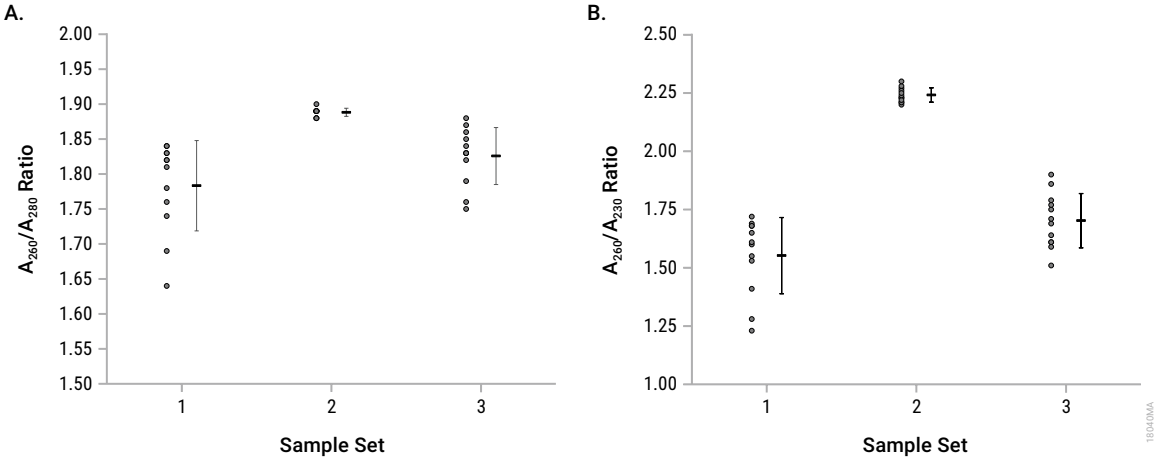


Figure 12. Tissue DNA quality. For 50mg heart, pancreas, and brain tissues with a 200µl elution volume, the average A_{260}/A_{280} ratios were in the range of 1.7–1.9 (**Panel A**) and the average A_{260}/A_{230} ratios were in the range of 1.5–2.3 (**Panel B**). In the graph, sample set 1 refers to heart tissue, sample set 2 refers to pancreas tissue, and sample set 3 refers to brain tissue.

Cells

For 5×10^6 HEK293 tissue culture cells with a 200µl elution volume, the average A_{260}/A_{280} ratio was 1.9 and the average A_{260}/A_{230} ratio was 2.3.

Cell Type	Input Cell Number	Elution Volume	A_{260}/A_{280}	A_{260}/A_{230}
HEK293 tissue culture cells	5×10^6	200µl	1.9	2.3
			1.9	2.3
			1.9	2.3
			1.9	2.2
			1.9	2.3
			1.9	2.3
			1.9	2.3
			1.9	2.2
			1.9	2.3
			1.9	2.3
			1.9	2.3
			1.9	2.3
		Average	1.9	2.3

Bone Marrow

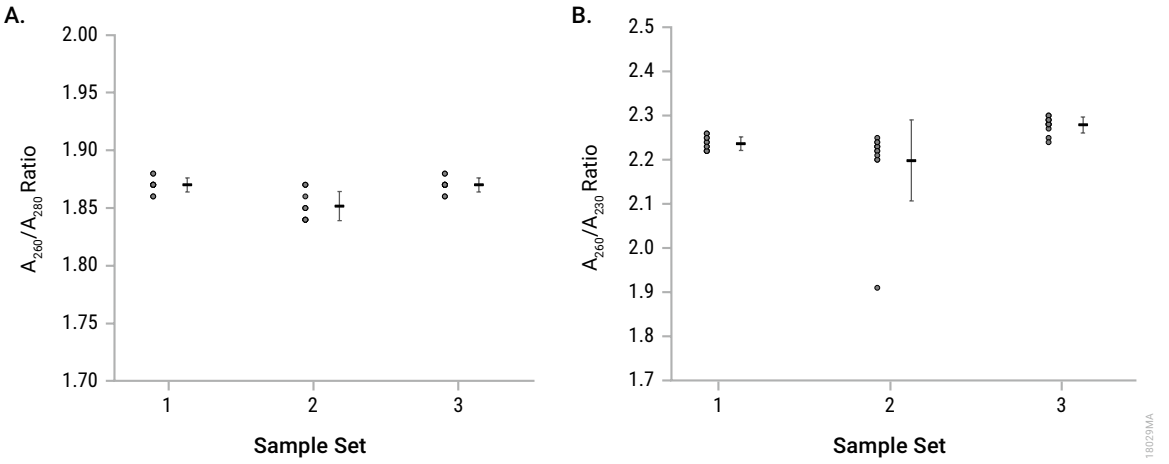


Figure 13. Bone marrow DNA quality. For 300µl bone marrow aspirates collected in EDTA, citrate and heparin tubes and with an elution volume of 200µl, the average A_{260}/A_{280} ratios were in the range of 1.8–1.9 (**Panel A**) and the average A_{260}/A_{230} ratios were in the range of 2.2–2.3 (**Panel B**). In the graph, sample set 1 refers to bone marrow aspirates collected in EDTA tubes, sample set 2 refers to bone marrow aspirates collected in citrate tubes, and sample set 3 refers to bone marrow aspirates collected in heparin tubes.

8.C. Reproducibility

To evaluate precision in DNA purification within each extraction run, DNA was purified from eight 300µl replicates of a single human whole blood sample during three instrument runs with Instrument 1 and four 300µl replicates of a single human whole blood sample during three instrument runs with Instrument 2. DNA yield was quantified by absorbance and the coefficient of variation (percent CV) was then calculated for each of the three runs for each instrument. DNA yield with the Maxwell® CSC Genomic DNA Kit was reproducible within each run, with intra-run percent CVs in the range of 6–9% for Instrument 1 and intra-run percent CVs in the range of 5–12% for Instrument 2.

To determine precision in DNA purification between extraction runs, DNA was purified from eight 300µl replicates of a single human whole blood sample during three instrument runs with Instrument 1 and four 300µl replicates of a single human whole blood sample during three instrument runs with Instrument 2. DNA yield was quantified by absorbance and the coefficient of variation (percent CV) was then calculated for all samples from all three runs for each instrument. DNA yield with the Maxwell® CSC Genomic DNA Kit was reproducible across runs, with 7% inter-run CV for Instrument 1 and an 8% inter-run CV for Instrument 2.

Instrument	Run #	Intra-Run Percent CV	Inter-Run Percent CV
1	1 (n = 8)	9%	7%
	2 (n = 8)	7%	
	3 (n = 8)	6%	
2	1 (n = 4)	12%	8%
	2 (n = 4)	7%	
	3 (n = 4)	5%	

8.D. Amplifiability

Compatibility with downstream amplification was assessed using DNA purified from fresh and frozen whole blood collected in EDTA tubes, frozen whole blood collected in citrate and heparin tubes, fresh and frozen buffy coat samples generated from whole blood collected in EDTA tubes, frozen buffy coat samples generated from whole blood collected in citrate and heparin tubes, 1 and 2 buccal swabs with and without preprocessing with a clearing column, heart, pancreas, and brain tissue, tissue culture cells, amniotic fluid, urine, and PBMCs, and frozen bone marrow aspirates collected in EDTA, citrate and heparin tubes with the Maxwell® CSC Genomic DNA Kit.

DNA purifications were performed for each sample type with the highest and lowest sample input amounts and elution volumes for each sample type. Tissue culture cells and PBMCs also included a cell number dilution series.

The resulting DNA from all samples were quantified by absorbance, diluted to a concentration within the qPCR standard curve and then amplified with a qPCR assay using the 300bp target. The C_q value for each purified DNA sample and the average C_q value for three replicates of the 0.0032ng/µl human genomic DNA standard supplied with the qPCR assay are reported.

The graphs in this section represent the C_q values of each replicate that was purified for each sample type. Each dot in the graphs represents an individual measurement on the left while the mean with standard deviation is on the right. Each data set includes a total of 12 replicates, four replicates purified using the Maxwell® CSC Instrument and eight replicates purified using the Maxwell® CSC 48 Instrument.

The tables below the figure legends describe the sample information for each sample set shown in the associated graphs.

Whole Blood

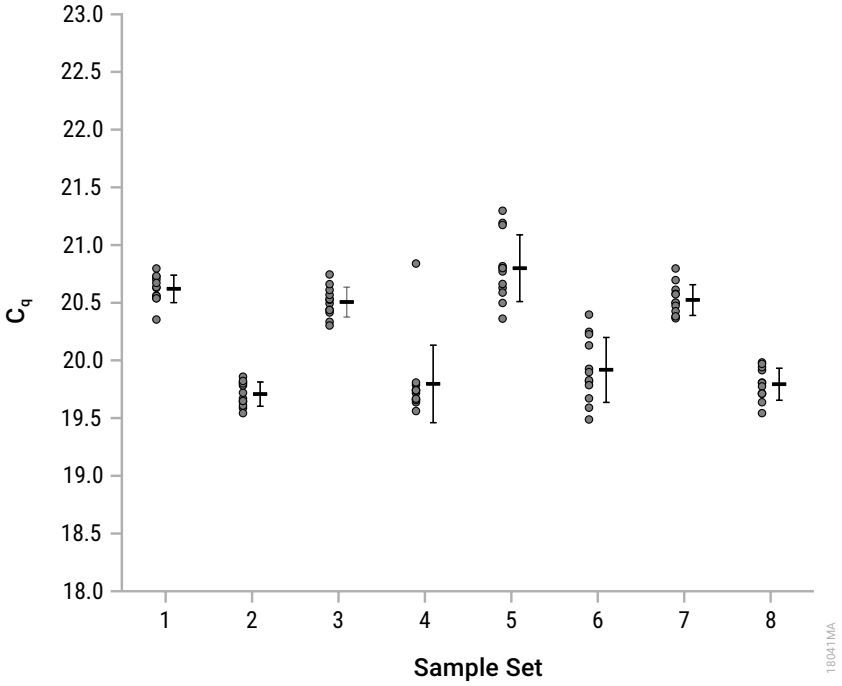


Figure 14. Whole blood DNA amplification. For fresh and frozen whole blood samples collected in EDTA tubes, C_q values ranged from 19.54–20.80 cycles, and all were well below the average C_q value for the 0.0032ng/µl DNA standard (33.11 cycles). For frozen whole blood samples collected in citrate and heparin tubes, C_q values ranged from 19.49–21.30 cycles, and all were well below the average C_q value for the 0.0032ng/µl DNA standard (32.88 cycles).

Sample Set	Anticoagulant	Storage	Input Volume (µl)	Elution Volume (µl)
1	EDTA	Frozen	50	50
2	EDTA	Frozen	300	200
3	EDTA	Fresh	50	50
4	EDTA	Fresh	300	200
5	Citrate	Frozen	50	50
6	Citrate	Frozen	300	200
7	Heparin	Frozen	50	50
8	Heparin	Frozen	300	200

8.D. Amplifiability (continued)

Buffy Coat

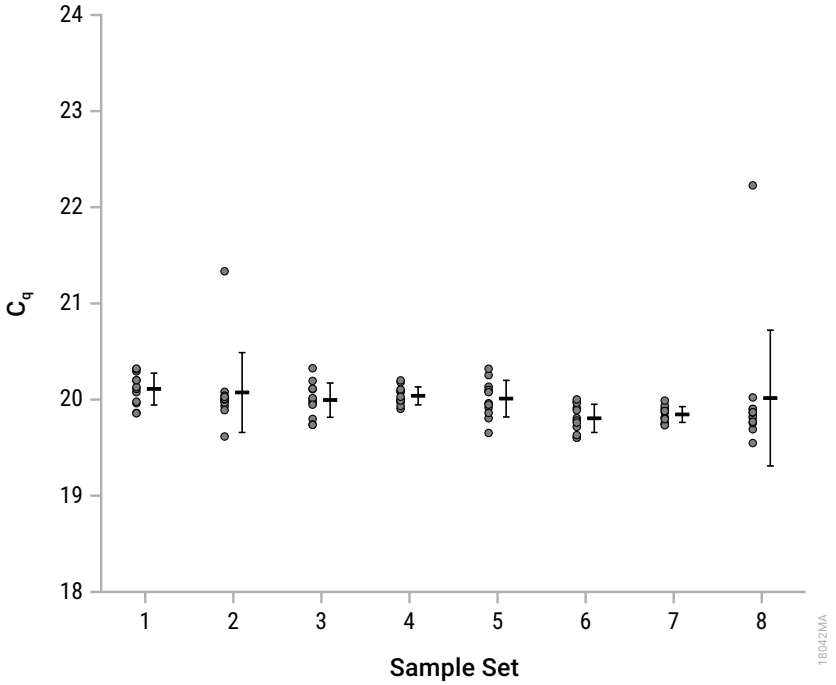


Figure 15. Buffy coat DNA amplification. For fresh and frozen buffy coat samples generated from whole blood collected in EDTA tubes, C_q values ranged from 19.62–21.34 cycles, and all were well below the average C_q value for the 0.0032ng/µl DNA standard (33.09 cycles). For frozen buffy coat samples generated from whole blood collected in citrate and heparin tubes, C_q values ranged from 19.55–22.23 cycles, and all were well below the average C_q value for the 0.0032ng/µl DNA standard (32.86 cycles).

Sample Set	Anticoagulant	Storage	Input Volume (µl)	Elution Volume (µl)
1	EDTA	Frozen	50	50
2	EDTA	Frozen	300	200
3	EDTA	Fresh	50	50
4	EDTA	Fresh	300	200
5	Citrate	Frozen	50	50
6	Citrate	Frozen	300	200
7	Heparin	Frozen	50	50
8	Heparin	Frozen	300	200

Buccal Swab

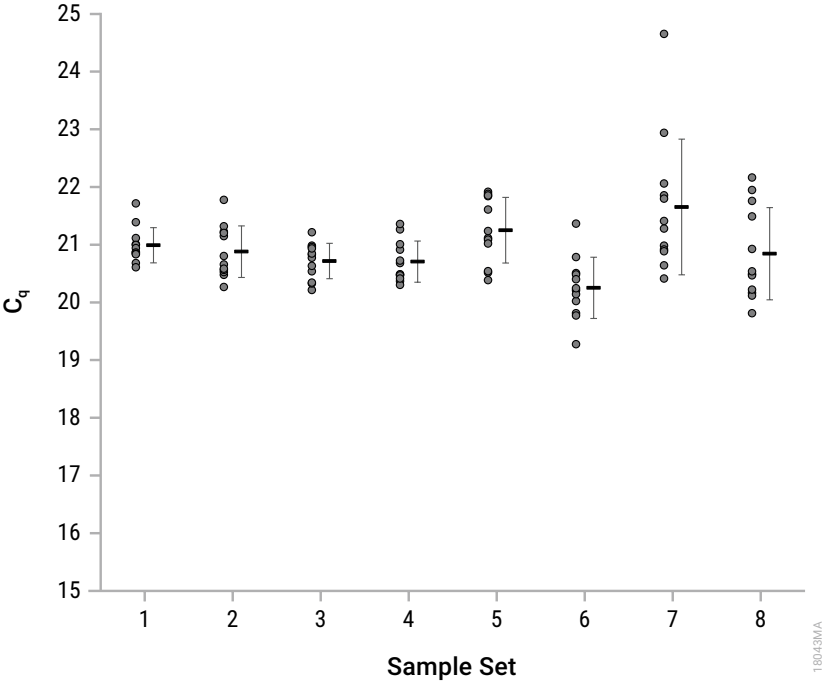


Figure 16. Buccal swab DNA amplification. For one and two input buccal swabs preprocessed with a clearing column, C_q ranged from 20.22–21.78 cycles, and all were well below the average C_q value for the 0.0032ng/µl DNA standard (32.45 cycles). For one and two input buccal swabs preprocessed without a clearing column, C_q values ranged from 19.28–24.65 cycles, and all were well below the average C_q value for the 0.0032ng/µl DNA standard (32.54 cycles).

Sample Set	Swab Number	Preprocessing	Elution Volume (µl)
1	1 swab	With Clearing Column	50
2	1 swab	With Clearing Column	200
3	2 swabs	With Clearing Column	50
4	2 swabs	With Clearing Column	200
5	1 swab	Without Clearing Column	50
6	1 swab	Without Clearing Column	200
7	2 swabs	Without Clearing Column	50
8	2 swabs	Without Clearing Column	200

8.D. Amplifiability (continued)

Tissue

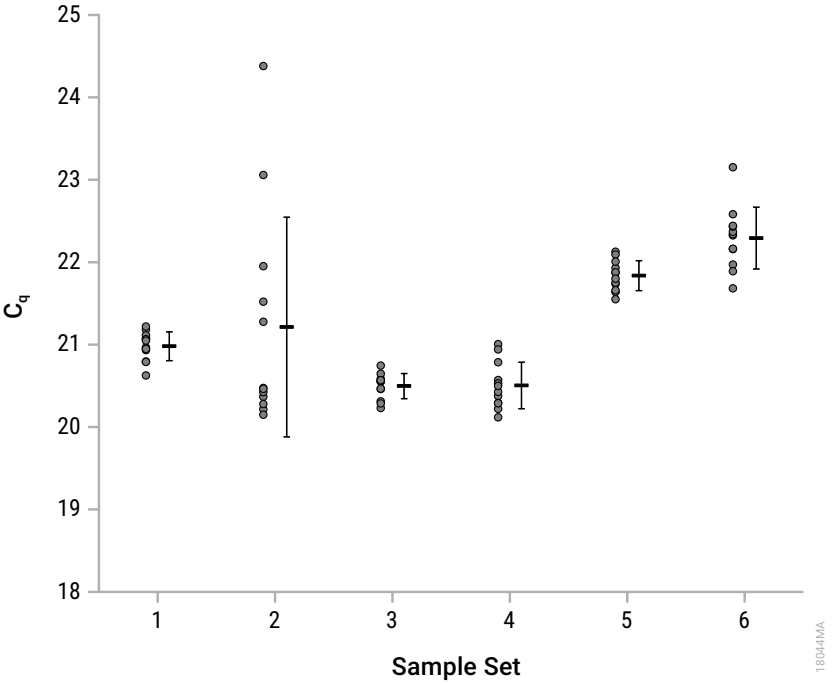


Figure 17. Tissue DNA amplification. For heart and pancreas tissues, C_q values ranged from 20.12–24.38 cycles, and all were well below the average C_q value for the 0.0032ng/µl DNA standard (32.98 cycles). For brain tissue, C_q values ranged from 21.55–23.15 cycles, and all were well below the average C_q value for the 0.0032ng/µl DNA standard (33.68 cycles).

Sample Set	Tissue Type	Input Quantity (mg)	Elution Volume (µl)
1	Heart	5	50
2	Heart	50	200
3	Pancreas	5	50
4	Pancreas	50	200
5	Brain	5	50
6	Brain	50	200

Cells

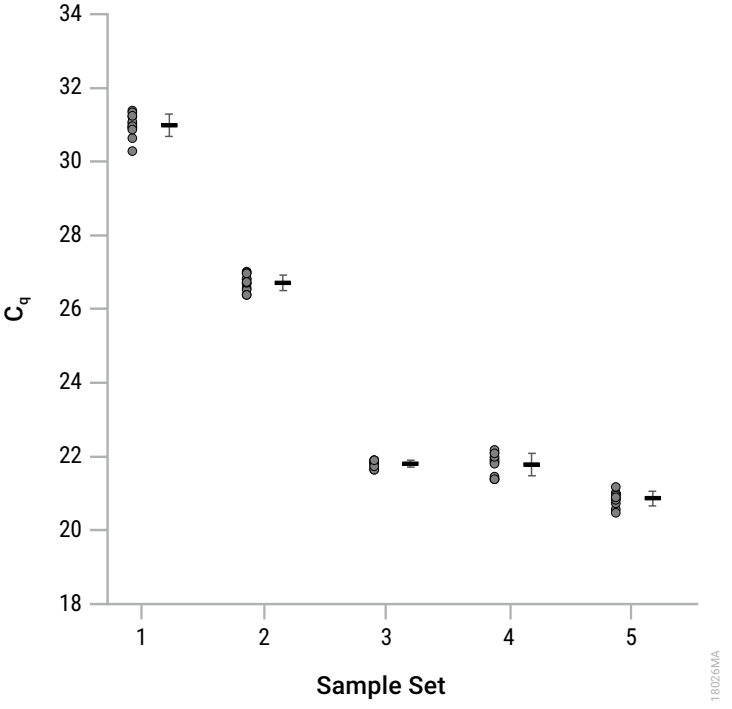


Figure 18. Tissue culture cell DNA amplification. For the HEK293 tissue culture cells dilution series, C_q values ranged from 20.48–31.38 cycles, and all were below the average C_q value for the 0.0032ng/µl DNA standard (33.04 cycles).

Sample Set	Cell Type	Cell Number	Elution Volume (µl)
1	HEK293 tissue culture cells	5×10^2	50
2	HEK293 tissue culture cells	5×10^3	50
3	HEK293 tissue culture cells	5×10^4	50
4	HEK293 tissue culture cells	5×10^5	200
5	HEK293 tissue culture cells	5×10^6	200

8.D. Amplifiability (continued)

Cells (continued)

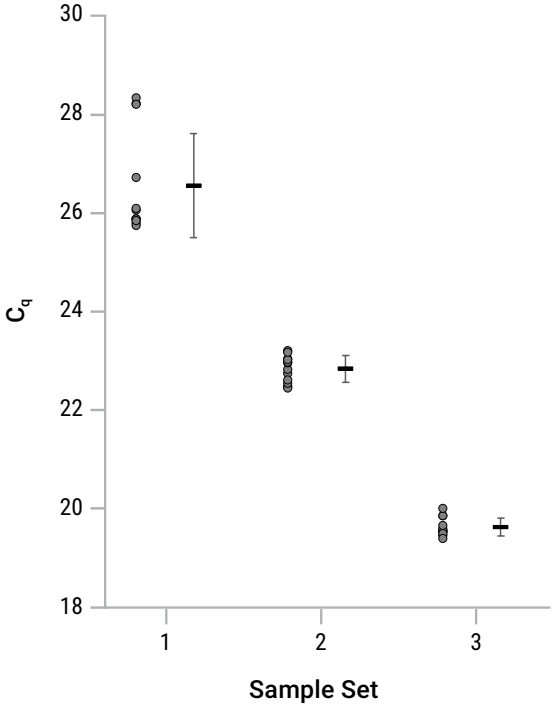


Figure 19. PBMC DNA amplification. For the PBMC dilution series, C_q values ranged from 19.40–28.33 cycles, and all were below the average C_q value for the 0.0032ng/µl DNA standard (32.80 cycles).

Sample Set	Cell Type	Cell Number	Elution Volume (µl)
1	PBMCs	5×10^4	50
2	PBMCs	5×10^5	100
3	PBMCs	5×10^6	200

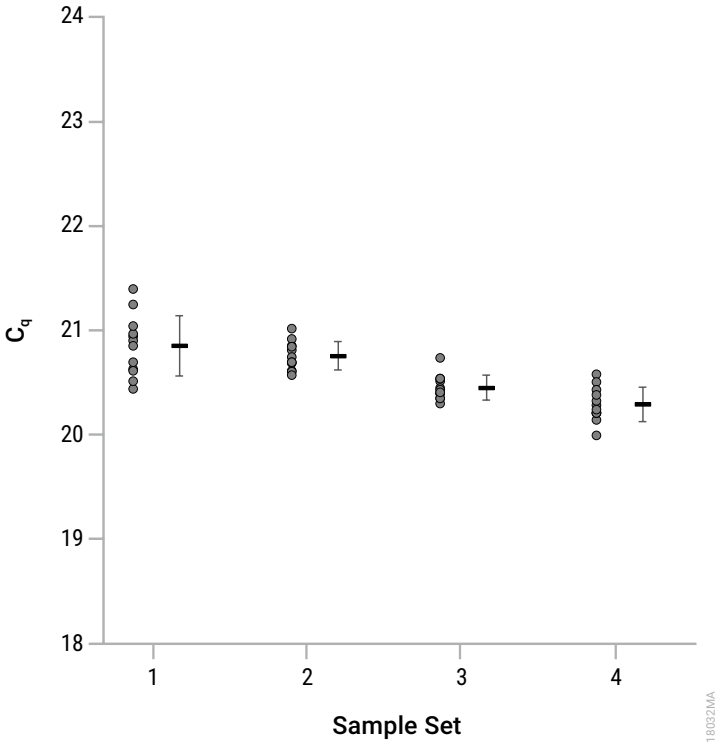


Figure 20. Urine and aminotic fluid DNA amplification. For cells obtained from urine and amniotic fluid samples, C_q values ranged from 20.00–21.40 cycles, and all were well below the average C_q value for the 0.0032ng/µl DNA standard (32.71 cycles).

Sample Set	Cell Type	Input Volume (ml)	Elution Volume (µl)
1	Urine	15	50
2	Urine	50	50
3	Amniotic fluid	1	50
4	Amniotic fluid	5	50

8.D. Amplifiability (continued)

Bone Marrow

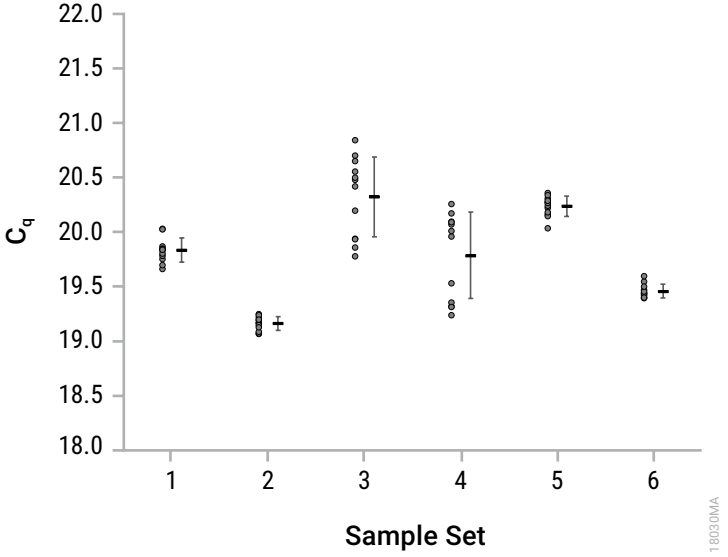


Figure 21. Bone marrow DNA amplification. For frozen bone marrow aspirates collected in EDTA and citrate tubes, C_q values ranged from 19.07–20.84 cycles, and all were well below the average C_q value for the 0.0032ng/µl DNA standard (32.42 cycles). For bone marrow aspirates collected in heparin tubes, C_q ranged from 19.40–20.36 cycles, and all were well below the average C_q value for the 0.0032ng/µl DNA standard (32.82 cycles).

Sample Set	Anticoagulant	Input Volume (µl)	Elution Volume (µl)
1	EDTA	50	50
2	EDTA	300	200
3	Citrate	50	50
4	Citrate	300	200
5	Heparin	50	50
6	Heparin	300	200

8.E. Inhibition (Interfering Substances)

Amplification inhibition was assessed using DNA purified from fresh and frozen whole blood collected in EDTA tubes, frozen whole blood collected in citrate and heparin tubes, fresh and frozen buffy coat samples generated from whole blood collected in EDTA tubes, frozen buffy coat samples generated from whole blood collected in citrate and heparin tubes, one and two buccal swabs with and without preprocessing with a clearing column, heart, pancreas, and brain tissues, tissue culture cells, amniotic fluid, urine, and PBMCs, and frozen bone marrow aspirates collected in EDTA, citrate and heparin tubes with the Maxwell® CSC Genomic DNA Kit.

DNA purifications were performed for each sample type. Sample input amounts and elution volumes in which the least amount of dilution would be needed for the samples to be used in the qPCR were assessed for this analysis.

The DNA was quantified and diluted to a concentration within the qPCR standard curve, and an aliquot of each DNA was then diluted an additional eightfold. The initial DNA dilutions and eightfold dilutions were amplified using a qPCR assay. The difference in C_q values ($|\Delta C_q|$) for the 300bp target sequence are reported. An $|\Delta C_q|$ of 3 ± 1 cycles corresponds to no inhibition of DNA amplification due to endogenous and exogenous substances that may be present in the samples.

The graphs in this section represent the $|\Delta C_q|$ of each replicate that was purified for each sample type. Each dot in the graphs represents an individual measurement on the left while the mean with standard deviation is on the right. Each data set includes a total of 12 replicates, four replicates purified using the Maxwell® CSC Instrument and eight replicates purified using the Maxwell® CSC 48 Instrument.

The tables below the figure legends describe the sample information for each sample set shown in the associated graphs.

8.E. Inhibition (Interfering Substances; continued)

Whole Blood

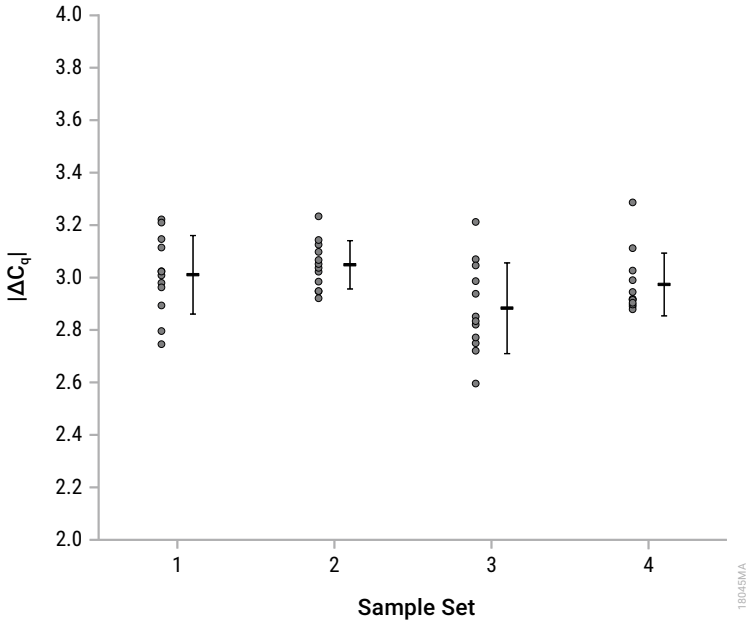


Figure 22. Test for inhibition in whole blood DNA amplification. For fresh and frozen whole blood samples collected in EDTA tubes, $|\Delta C_q|$ values ranged from a low of 2.75 cycles to a high of 3.23 cycles. For frozen whole blood samples collected in citrate and heparin tubes, $|\Delta C_q|$ values ranged from a low of 2.60 cycles to a high of 3.29 cycles.

Sample Set	Anticoagulant	Storage	Input Volume (µl)	Elution Volume (µl)
1	EDTA	Frozen	50	50
2	EDTA	Fresh	50	50
3	Citrate	Frozen	50	50
4	Heparin	Frozen	50	50

Buffy Coat

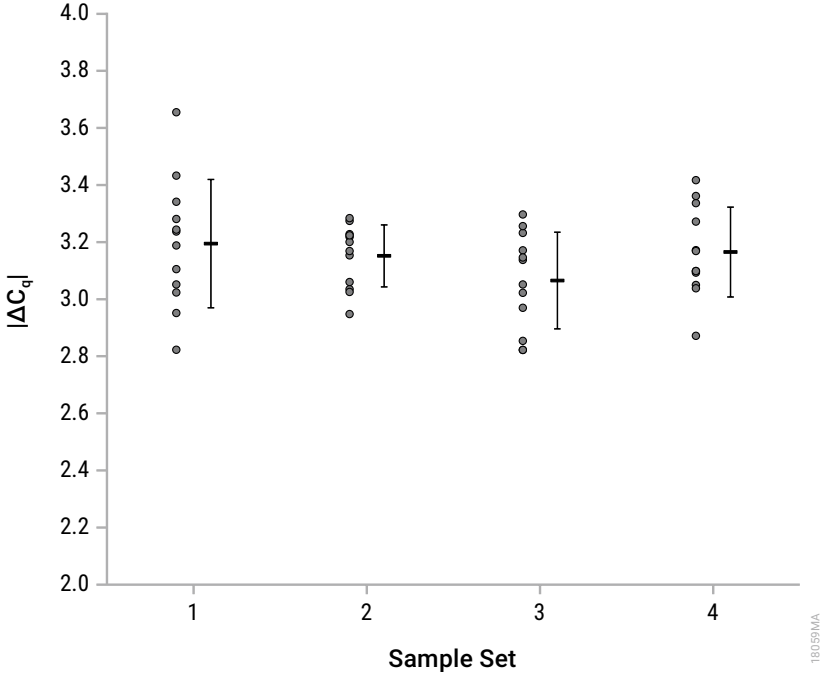


Figure 23. Test for inhibition in buffy coat DNA amplification. For fresh and frozen buffy coat samples generated from whole blood collected in EDTA tubes, $|\Delta C_q|$ values ranged from a low of 2.82 cycles to a high of 3.65 cycles. For frozen buffy coat samples generated from whole blood collected in citrate and heparin tubes, $|\Delta C_q|$ values ranged from a low of 2.82 cycles to a high of 3.42 cycles.

Sample Set	Anticoagulant	Storage	Input Volume (µl)	Elution Volume (µl)
1	EDTA	Frozen	50	50
2	EDTA	Fresh	50	50
3	Citrate	Frozen	50	50
4	Heparin	Frozen	50	50

8.E. Inhibition (Interfering Substances; continued)

Buccal Swab

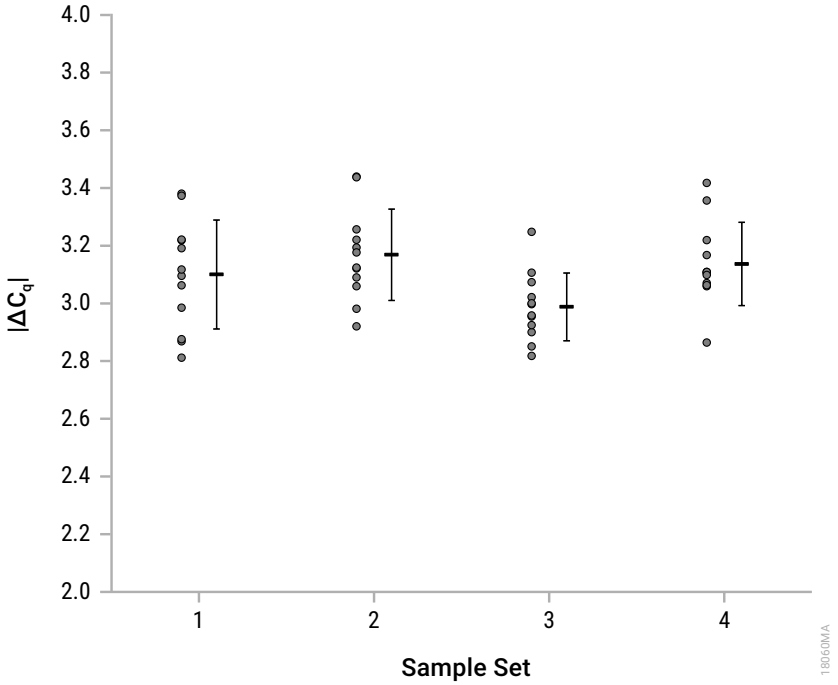


Figure 24. Test for inhibition in buccal swab DNA amplification. For one and two input buccal swabs preprocessed with a clearing column, $|\Delta C_q|$ values ranged from a low of 2.81 cycles to a high of 3.44 cycles. For one and two input buccal swabs preprocessed without a clearing column, $|\Delta C_q|$ values ranged from a low of 2.82 cycles to a high of 3.42 cycles.

Sample Set	Swab Number	Preprocessing	Elution Volume (µl)
1	1 swab	With Clearing Column	50
2	2 swabs	With Clearing Column	50
3	1 swab	Without Clearing Column	50
4	2 swabs	Without Clearing Column	50

Tissue

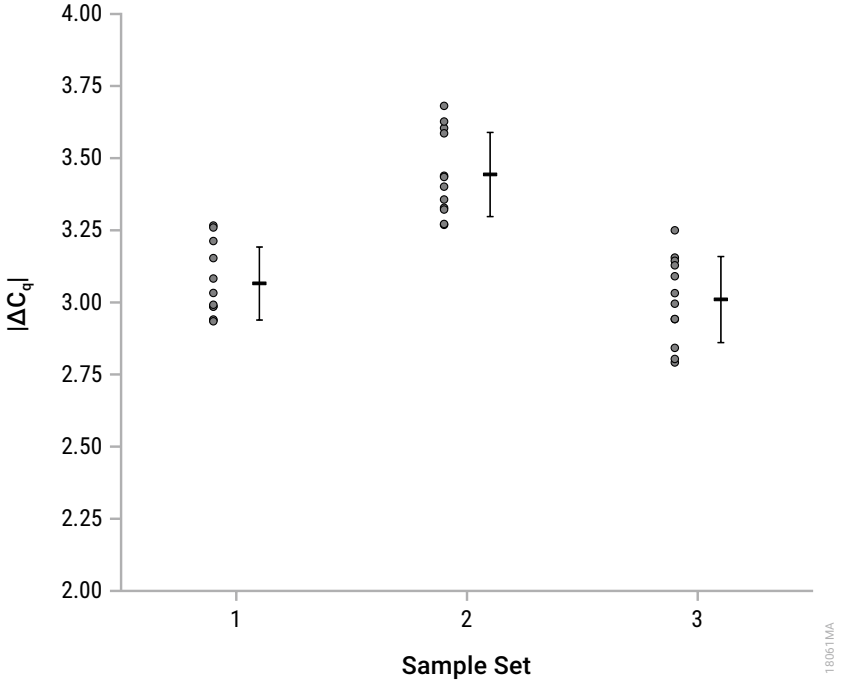


Figure 25. Test for inhibition in tissue DNA amplification. For 5mg heart and pancreas tissues, $|\Delta C_q|$ values ranged from a low of 2.93 cycles to a high of 3.68 cycles. For 5mg brain tissue, $|\Delta C_q|$ values ranged from a low of 2.79 cycles to a high of 3.25 cycles. An elution volume of 50µl was used for all samples. In the graph, sample set 1 refers to heart tissue, sample set 2 refers to pancreas tissue, and sample set 3 refers to brain tissue.

8.E. Inhibition (Interfering Substances; continued)

Cells

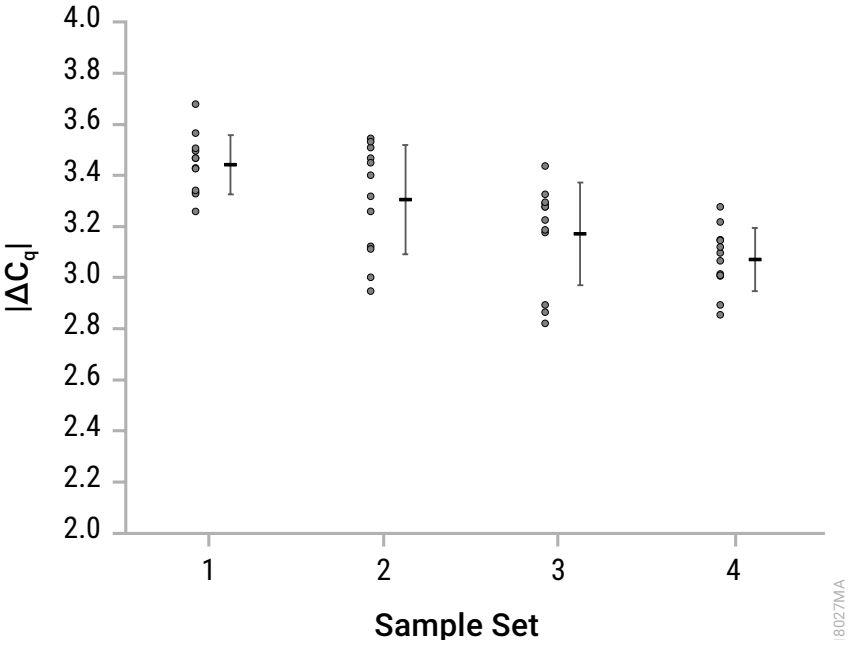


Figure 26. Test for inhibition in cell DNA amplification. For 5×10^4 HEK293 tissue culture cells with an elution volume of 50µl, $|\Delta C_q|$ values ranged from a low of 3.26 cycles to a high of 3.68 cycles. For 5×10^5 PBMCs with an elution volume of 100µl, $|\Delta C_q|$ values ranged from a low of 2.95 cycles to a high of 3.55 cycles. For cells obtained from 50ml urine and 5ml amniotic fluid samples with an elution volume of 50µl, $|\Delta C_q|$ values ranged from a low of 2.82 cycles to a high of 3.44 cycles.

Sample Set	Cell Type	Input Quantity	Elution Volume (µl)
1	HEK293 tissue culture cells	5×10^4 cells	50
2	PBMCs	5×10^5 cells	100
3	Urine	50ml	50
4	Amniotic fluid	5ml	50

Bone Marrow

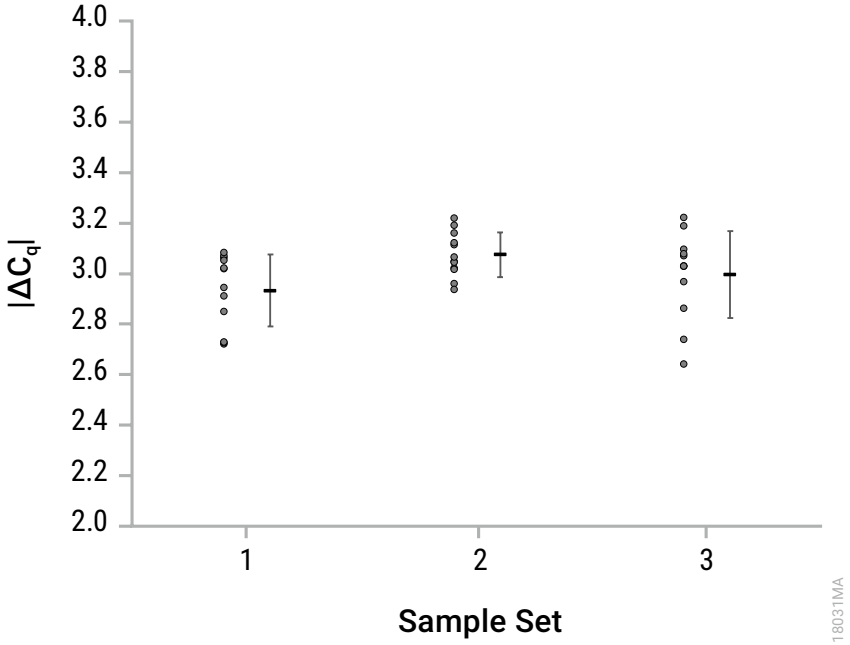


Figure 27. Test for inhibition in bone marrow DNA amplification. For 50µl bone marrow aspirates collected in EDTA and citrate tubes, $|\Delta C_q|$ values ranged from a low of 2.72 cycles to a high of 3.22 cycles. For 50µl bone marrow aspirates collected in heparin tubes, $|\Delta C_q|$ values ranged from a low of 2.64 cycles to a high of 3.22 cycles. An elution volume of 50µl was used for all samples. In the graph, sample set 1 refers to bone marrow aspirates collected in EDTA tubes, sample set 2 refers to bone marrow aspirates collected in citrate tubes, and sample set 3 refers to bone marrow aspirates collected in heparin tubes.

8.F. Cross-contamination

Male (300µl) and female (50µl) buffy coat samples were processed in alternating deck positions of the Maxwell® instruments, and the resulting purified female DNA samples were amplified using a Y chromosomal DNA target with a qPCR assay. The presence of this Y chromosomal target in the female samples was used to identify potential cross-contamination from neighboring samples. When female buffy coat samples were processed in deck positions adjacent to male buffy coat samples, no female DNA samples exhibited a C_q value for the Y chromosomal DNA target.

9. Clinical Performance Evaluation

The clinical performance evaluation was performed using human samples with the Maxwell® CSC Genomic DNA Kit and the Maxwell® CSC 48 Instrument.

Whole Blood

Two testers from an external laboratory purified DNA from 200µl human whole blood samples with an elution volume of 100µl from 12 individual donors with the Maxwell® CSC purification system as well as a laboratory reference extraction method. The resulting eluates were analyzed by amplification of the positive control HCP5 housekeeping gene in the HLA-B27 assay. All 12 DNA samples purified with the Maxwell® CSC Genomic DNA Kit demonstrated concordance between the two testers and to the laboratory reference extraction method.

Buccal Swab

One tester from an external laboratory purified DNA from one human buccal swab sample preprocessed with a clearing column and an elution volume of 100µl from 12 individual donors with the Maxwell® CSC purification system as well as a laboratory reference extraction method. The resulting eluates were analyzed by amplification of the positive control HCP5 housekeeping gene in the HLA-B27 assay. All 12 DNA samples purified with the Maxwell® CSC Genomic DNA Kit demonstrated concordance to the laboratory reference extraction method.

Tissue

One tester from an external laboratory purified DNA from 10–25mg of human tissue samples with an elution volume of 200µl from 12 individual donors with the Maxwell® CSC purification system as well as a laboratory reference extraction method. The resulting eluates were analyzed by amplification of the positive control HCP5 housekeeping gene in the HLA-B27 assay. All 12 DNA samples purified with the Maxwell® CSC Genomic DNA Kit demonstrated concordance to the laboratory reference extraction method.

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 DNA concentration	Samples that have undergone multiple freeze-thaw cycles may have degraded DNA. Sample guidelines for collection and storage are listed for each specific sample type.
	Sample contained a low amount of genomic DNA. The genomic DNA yield depends on the amount of sample being processed and the DNA content of that sample.
	Proteinase K Solution was not added, an incomplete volume of Proteinase K Solution was added, or the Proteinase K was not effectively mixed with the sample. Lysis and yield are dependent upon complete extraction with Proteinase K.
	Input sample was not mixed before processing. Be sure to mix samples before processing.
	Elution volume used for extraction was too large for the sample being processed. To increase eluted DNA concentration, reduce the initial elution buffer volume.
	Too much sample or sample containing an excessive amount of genomic DNA was processed. Excessive sample or genomic DNA may cause extraction chemistry failure, resulting in an eluate concentration that does not correlate with the amount of sample being processed.
	Lysate was not mixed with the binding solution in well #1 by aspirating and dispensing 5–10 times after transfer to make a homogeneous mixture. Failure to create a homogeneous mixture of sample lysate and binding solution in well #1 can result in decreased yield and purity in the final eluate.
	Samples were not mixed appropriately or at the correct steps during processing. Failure to adequately mix reagents and samples together in the incubation tube can affect performance.
	Lysis Buffer and Lytic Enhancer (LE2) were used interchangeably, at the incorrect step or at the incorrect volume. Reprocess samples, correctly using Lysis Buffer and Lytic Enhancer (LE2) as instructed.

10. Troubleshooting (continued)

Symptoms	Causes and Comments
Lower than expected purity	Proteinase K Solution was not added, an incomplete volume of Proteinase K Solution was added, or the Proteinase K was not effectively mixed with the sample. Lysis and yield are dependent upon complete extraction with Proteinase K.
	Lysate was not mixed with the binding solution in well #1 by aspirating and dispensing 5–10 times after transfer to make a homogeneous mixture. Failure to create a homogeneous mixture of sample lysate and binding solution in well #1 can result in decreased yield and purity in the final eluate.
	Samples that have undergone multiple freeze-thaw cycles may have degraded DNA. Use samples that have been collected and stored under the guidelines listed under each specific sample type.
	For whole blood, buffy coat, and bone marrow samples, transferring clotty or fatty material into the incubation tube can result in poor lysis of the sample. Transfer only liquid samples for purification.
	Lysis Buffer and Lytic Enhancer (LE2) were used interchangeably, at the incorrect step or at the incorrect volume. Reprocess samples, correctly using Lysis Buffer and Lytic Enhancer (LE2) as instructed.
	Some tissue types can produce lower than expected purity values. If higher purity values are desired, reduce the input amount of tissue processed.
	Samples were not mixed appropriately or at the correct steps during processing. Failure to adequately mix reagents and samples together in the incubation tube can affect performance.
	Transferring solid material into well #1 of the cartridge can result in copurification of solid material and contaminants. Remove solid material before transferring lysed sample into the cartridge.
RNA contamination	RNase A Solution was not added to well #3 of the cartridge or an incomplete volume of RNase A Solution was added. Reprocess sample with RNase A Solution or treat extracted gDNA sample with RNase A.

Symptoms	Causes and Comments
Resin carryover	Samples were not mixed appropriately or during the correct steps during processing. Failure to adequately mix reagents and samples together in the incubation tube or well #1 can affect resin carryover in the cartridge and elution tube.
	Too much sample or sample containing an excessive amount of genomic DNA was processed. Excessive sample may cause excess resin carryover in the cartridge and elution tube.
	Some resin carryover is normal and does not affect downstream performance. If necessary, use an Elution Magnet ([Cat.# AS4017, Cat.# AS4018 or both]; available separately) to transfer the eluate into a new tube. See Section 11, Related Products.

11. Related Products

Instruments and Accessories

Product	Size	Cat.#
Maxwell® CSC 48 Instrument*	1 each	AS8000
Maxwell® CSC Instrument*	1 each	AS6000
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
RSC/CSC Plungers	50/pack	AS1331
Elution Tubes (0.5ml)	50/pack	AS6201
Elution Magnet, 16 Position	1 each	AS4017
Elution Magnet, 24 Position	1 each	AS4018
Clearing Columns	50 each	Z3871
RNase A Solution	1ml	A7973
	5ml	A7974
Proteinase K (PK) Solution	4ml	MC5005
Nuclease-Free Water	25ml	MC1191

*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.

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

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Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

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