



TECHNICAL MANUAL

Maxwell[®] DE System

Instructions for Use of Product
AS1870

Maxwell[®] DE System

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

1. Description	2
2. Product Components and Storage Conditions	2
3. Before You Begin	3
4. Sample Preprocessing	3
4.A. Preparing Diluted 1-Thioglycerol	3
4.B. Preparing Maxwell [®] DE Lysis Mix	4
4.C. Preparing Maxwell [®] DE Elution Mix	4
4.D. Preprocessing Samples using Spin Baskets and ClickFit Microtubes, 1.5ml	5
5. Preparing the Maxwell[®] DE System Cartridge for Use with the Maxwell[®] FSC, Maxwell[®] RSC or Maxwell[®] RSC 48 Instrument	6
6. Maxwell[®] Instrument Run	8
7. Storing and Using Non-Sperm and Sperm Fractions in Downstream Applications	10
7.A. Recommendations for Using the Non-Sperm Fraction	10
7.B. Recommendations for Using the PowerQuant [®] System	10
7.C. Recommendations for Using the Plexor [®] HY System	11
7.D. Recommendations for Using the PowerPlex [®] ESI Fast and ESX Fast Systems	11
7.E. Amplifying with PowerPlex [®] Systems That Do Not Require 5X AmpSolution [™] Reagent	12
7.F. Recommendations for Using Other Quantification and STR Systems and Applications	13
8. Troubleshooting	14
9. Related Products	15
10. Summary of Changes	16

1. Description

Sexual assault evidence kit (SAEK) samples are commonly comprised of a mixture of non-sperm and sperm cells from multiple individuals. DNA enrichment from sperm cells can improve the chances of obtaining a usable and interpretable short tandem repeat (STR) profile for the sperm contributor.

The Maxwell® DE System^(a) is a differential extraction system designed specifically for forensic laboratories. This system employs novel paramagnetic particles to generate sperm and non-sperm fractions from mixed biological samples, such as those commonly found in SAEKs, for use in downstream PCR quantification and STR analysis applications.

Throughout this manual, the terms non-sperm fraction and sperm fraction are used to describe the separated cell fractions generated during differential extraction. These labels are intended to describe processing paths and do not imply confirmed biological content. In some laboratory workflows, these are also referred to as Fraction 1 (non-sperm) and Fraction 2 (sperm).

2. Product Components and Storage Conditions

PRODUCT	SIZE	CAT. #
Maxwell® DE System	48 preps	AS1870

Not For Medical Diagnostic Use. Sufficient for 48 automated isolations from forensic casework samples. Includes:

- 900µl 1-Thioglycerol
- 4 × 1ml Proteinase K
- 60ml Maxwell® DE Resuspension Buffer
- 20ml Maxwell® DE Elution Buffer
- 30ml Maxwell® DE Lysis Buffer
- 25ml Nuclease-Free Water
- 48 Maxwell® DE Cartridges
- 48 Maxwell® RSC Plunger Pack
- 50 Elution Tubes, 0.5ml
- 50 DNA IQ™ Spin Baskets
- 100 ClickFit Microtube, 1.5 ml

Storage Conditions: Store the components of the Maxwell® DE System at +15°C to +30°C. Upon receipt, store 1-Thioglycerol at +2°C to +10°C.



Safety Information: The Maxwell® DE Cartridges contain the flammable substance ethanol. 1-Thioglycerol is a toxic substance. We highly recommend the use of gloves and aerosol-resistant pipette tips.



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



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

Additional Information: Do not use cartridges if the seal on the cartridge is not intact on receipt. For additional safety information, see the Safety Data Sheet, available at: www.promega.com

3. Before You Begin

The quality of a differential extraction from any sample can be influenced by sample age, substrate and environmental storage conditions. The Maxwell® DE System should be evaluated as part of a laboratory's internal validation. The Maxwell® DE System has not been tested with all possible biological fluids or substrates. Nonstandard samples may require additional evaluation to ensure reliable performance.

Materials to Be Supplied By the User

- Maxwell® FSC (Cat.# AS4600), Maxwell® RSC (Cat.# AS4500), or Maxwell® RSC 48 (Cat.# AS8500) Instrument with the method installed for the Maxwell® DE System.
If needed, the method can be downloaded at:
www.promega.com/resources/software-firmware/maxwell-maxprep/maxwell-rsc-fsc-software-firmware-methods/
or
www.promega.com/resources/software-firmware/maxwell-maxprep/maxwell-rsc-48-software-firmware-methods/
- 56°C heat block or water bath, compatible with 1.5ml tubes
- 70°C heat block or water bath, compatible with 0.5ml tubes
- aerosol-resistant pipette tips
- microcentrifuge with rotor compatible with 1.5ml ClickFit tubes and capable of 16,000–20,000 × g
- **optional:** 95°C heat block or water bath, compatible with 1.5ml tubes

4. Sample Preprocessing

The instructions in this section describe the manual steps necessary for preprocessing samples, including those on a solid support, such as swabs or fabric, in individual tubes. This preprocessing protocol can differentially extract from these samples.

4.A. Preparing Diluted 1-Thioglycerol

Add 5µl of 1-Thioglycerol to 45µl of Nuclease-Free Water. Vortex for 10 seconds at maximum speed to thoroughly mix.

Notes:

- 1-Thioglycerol is viscous. Pipette slowly for accuracy. Use the recommended dilution and do not pipet less than 5µl for accuracy.
- Dilute 1-Thioglycerol fresh for each use. Do **not** store the dilution.
- During longer preprocessing incubations such as when incubating overnight (e.g., Section 4.D, Step 4), we recommend preparing the 1-thioglycerol dilution near the end of the preprocessing heated incubation step.
- This volume of diluted 1-Thioglycerol is enough for up to 50 samples. Scale up the dilution for additional samples.

4.B. Preparing Maxwell® DE Lysis Mix

1. Determine the number of extractions to be performed. Increase this number by 1 or 2 to compensate for pipetting error.
2. Use Table 1 to calculate each component volume required to prepare sufficient Maxwell® DE lysis mix for the extraction number determined in Step 1.

Table 1. Preparing Lysis Mix.

Reagent	Volume per Extraction	×	Number of Extractions	=	Final Volume
Maxwell® DE Lysis Buffer	390µl	×		=	
Proteinase K	60µl	×		=	

Note: Do not store this dilution; prepare fresh Maxwell® DE lysis mix for each use.

3. Vortex for 10 seconds at maximum speed to thoroughly mix.

4.C. Preparing Maxwell® DE Elution Mix

1. Determine the number of extractions to be performed. Increase this number by 1 or 2 to compensate for pipetting error.
2. Use Table 2 to calculate each component volume required to prepare sufficient Maxwell® DE elution mix for the extraction number determined in Step 1.

Table 2. Preparing Elution Mix.

Reagent	Volume per Extraction	×	Number of Extractions	=	Final Volume
Maxwell® DE Elution Buffer	198µl	×		=	
Proteinase K	1µl	×		=	
Diluted 1-Thioglycerol, (prepared in Section 4.A)	1µl	×		=	

Notes:

- a. Do not store this dilution; prepare fresh Maxwell® DE elution mix for each use.
 - b. During longer preprocessing incubations such as when incubating overnight (e.g., Section 4.D, Step 4), we recommend preparing the Maxwell® DE elution mix near the end of the preprocessing heated incubation step.
3. Vortex for 10 seconds at maximum speed to thoroughly mix.

4.D. Preprocessing Samples using Spin Baskets and ClickFit Microtubes, 1.5ml

1. Place a solid substrate at the bottom of a labeled ClickFit Microtube.
2. Add 450µl of Maxwell® DE lysis mix (prepared in Section 4.B) to each tube, ensuring that the substrate is completely covered.
3. Close the lid of each tube and vortex samples at maximum speed for 10 seconds.
4. Place the samples in a 56°C heat block or water bath and incubate for at least 4 hours up to overnight.

Notes:

- a. Do not use an incubator to incubate tubes. Heat transfer is inefficient and will result in poor performance.
- b. For certain samples, such as those expected to have higher quantities of sperm cells, incubating for as little as 2 hours at 56°C may be sufficient for effective sperm fraction separation. Do not incubate for less than 2 hours.
5. After the 56°C incubation, use clean forceps to carefully transfer solid substrate from the ClickFit Microtube (leaving lysis mix behind) to a clean DNA IQ™ Spin Basket, then place the DNA IQ™ Spin Basket containing the solid substrate back into the same ClickFit Microtube. Close the tube lid.

Notes:

- a. Alternatively, for each sample processed, place a DNA IQ™ Spin Basket into a clean, labeled ClickFit Microtube. Carefully transfer the substrate to the spin basket in the tube with the corresponding label. Transfer the liquid lysate from the incubation tube to the same spin basket and close the tube. Avoid pelleting sperm cells prior to transferring the liquid, such as by centrifugation, as sperm cells might be left behind. Additional ClickFit Microtubes are available for purchase (see Section 9).
- b. Carefully open the tubes as some condensation may be present on the caps of the tubes following incubation. Briefly centrifuging at low-speed or flicking the tubes may help remove this condensation.
- c. Only use the DNA IQ™ Spin Baskets that were provided with the Maxwell® DE System; other spin baskets (e.g., CW Spin Baskets Cat.# AS8101) are not compatible with the Maxwell® DE System. Should additional DNA IQ™ Spin Baskets be needed, they are available for purchase (see Section 9).
6. Centrifuge at room temperature for 5 minutes at maximum speed in a microcentrifuge (~16,000–20,000 × g). Carefully remove the Spin Basket without disrupting the pellet; the Spin Basket may be discarded after removal subject to laboratory policies. Transfer the supernatant (non-sperm fraction) to a clean, labeled ClickFit Microtube, 1.5ml, leaving about 50µl of supernatant and the entire pellet behind (sperm fraction). Close the caps of the tubes that contain the non-sperm fraction. The non-sperm fraction requires further processing before downstream use. See Section 7.A for further instructions.
7. Add 900µl of Maxwell® DE Resuspension Buffer to the sperm fraction. Resuspend pellet by either capping the tube and vortexing or vigorously pipetting up and down.
8. The sample is now ready for automated differential extraction using the Maxwell® Instrument.

5. Preparing the Maxwell® DE System Cartridge for Use with the Maxwell® FSC, Maxwell® RSC or Maxwell® RSC 48 Instrument

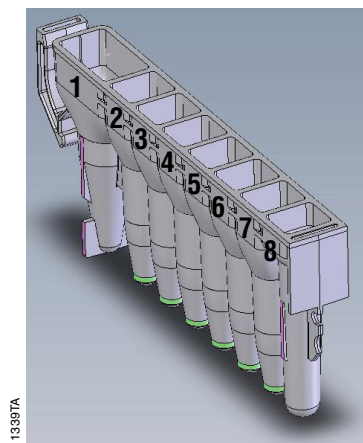
Table 3. Maxwell® Instruments and Associated Manuals. For detailed usage information, refer to the operating manual specific to your Maxwell® Instrument:

Instrument	Operating Manual
Maxwell® RSC	TM411
Maxwell® RSC 48	TM510
Maxwell® FSC	TM462

Before beginning purification, ensure that the Maxwell DE System method has been loaded onto the Maxwell® Instrument. Please see the section titled “Importing and Deleting Methods” in the relevant Maxwell® operating manual (Table 3) for further instructions on how to load method files. Also ensure that the Maxwell® DE elution mix has been freshly prepared and is ready to use (Section 4.C).

1. Change gloves before handling Cartridges, Plungers and Elution Tubes. Place the appropriate number of cartridges in the Maxwell® Cartridge Rack. Press down on both ends of the cartridge to snap it into position.
2. Carefully peel the seal off the Maxwell cartridges. Rotate the cartridge rack so that well #8 is closest to you. Well #8 is the position closest to the Elution Tube (Figure 1).

Note: Clean sample or reagent spills on any part of the Maxwell® Cartridge Rack with a detergent-water solution, followed by 70% ethanol, then water. Do not use bleach on any instrument parts.



User Adds to Wells

1. Processed sample
8. Plunger

Figure 1. Maxwell® DE Cartridge.

3. Place a Plunger into well #8 of each cartridge (Figure 1).

Note: Only use the Plungers provided with the Maxwell® DE System; other plungers are not compatible with the Maxwell® DE System.

4. Place labeled Elution Tubes in the Maxwell® Cartridge Rack with the caps facing away from the Maxwell® Cartridges (Figure 2). Add 200µl of Maxwell® DE elution mix (prepared in Section 4.C) to each tube.

Notes:

- a. Make sure that the Elution Tubes are pressed all the way down.
- b. Some evaporation will occur during elution. Using smaller volumes of Maxwell® DE elution mix could affect DNA recovery and risks completely drying the sample.
- c. Ensure that the Maxwell® DE elution mix is in the bottom of the tube. If Maxwell® DE elution mix is on the side of the tube, the elution may be suboptimal.
- d. Use only the Elution Tubes provided in this kit and the Maxwell® DE elution mix prepared in Section 4.C of this protocol.
- e. Ensure that the Maxwell® DE elution mix was prepared fresh on the same day as the Maxwell® instrument run.



Figure 2. Setup and configuration of the deck tray. Maxwell® DE elution mix is added to the elution tubes as shown. Plungers are in well #8 of the cartridge.

5. Change gloves again. Transfer the entire contents of each sample tube containing the resuspended Sperm Fraction (Section 4.D, Step 8) into well #1 of each Maxwell® cartridge (Figure 1).
6. Proceed to Section 6 to run the Maxwell® DE System method.

6. Maxwell® Instrument Run

The Maxwell® DE System method takes approximately 70 minutes to run. For detailed information, refer to the Operating Manual specific to your Maxwell® Instrument. See Table 3 (Section 5).

1. Turn on the Maxwell® Instrument and Tablet PC. Log into the Tablet PC and start the Maxwell® 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. On the 'Methods' screen, select a method using one of the two options below:
 - a. Touch the **Maxwell DE System** method from the list.
 - b. Use a bar code reader to scan the 2D bar code on the kit box to automatically select the appropriate method.
4. Verify that the Maxwell DE System method has been selected, and touch the **Proceed** button. If requested by the software, enter any kit lot and expiration information that has been required by the Administrator.
5. On the 'Cartridge Setup' screen, confirm that the Maxwell DE System 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 touch the **Proceed** button to continue.

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

6. 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 mix, 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.

7. Touch the **Start** button to begin the method. 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.

8. The Maxwell® Instrument will immediately proceed with the Maxwell® DE System run. The screen will display the steps performed and the approximate time remaining in the run.

Notes:

- a. Pressing the **Abort** button will abandon the run.
 - b. If the run is abandoned before completion, you may be prompted to check whether plungers are still loaded on the plunger bar. If plungers are present on the plunger bar, you should perform **Clean Up** when requested. If plungers are not present on the plunger bar, you can choose to skip **Clean Up** when requested. The samples will be lost.
9. When the automated Maxwell® DE System run is complete, the tablet screen will display a message that the method has ended.

Notes:

- a. We do not recommend leaving the finished eluate sitting in the Maxwell® Instrument as additional evaporation may occur.
- b. Some evaporation will occur during the elution step.

End of Run

10. Follow the on-screen instructions at the end of the method to open the instrument door. Verify that the plungers are located in well #8 of the cartridges. If plungers are not removed from the plunger bar, follow the instructions in the technical manual appropriate to your Maxwell® Instrument (see Table 3) to perform a Clean Up process to attempt to unload the plungers.
11. Remove the deck tray(s) from the instrument immediately following the run to prevent evaporation of the eluates. Close the Elution Tubes containing the sperm fraction, and remove them.

Note: Following the automated differential extraction 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.



12. 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® DE Cartridges, DE Plungers or Elution Tubes.
13. Place the closed Elution Tubes containing the sperm fractions into a 70°C heat block or water bath and incubate for 20 minutes.

Notes:

- a. The sperm fraction can be stored at -30°C to +10°C for at least 3 months.
- b. The sperm fraction is directly compatible with Promega short-tandem repeat (STR) chemistries, including the PowerQuant® and PowerPlex® systems (see Section 7 for a full list of recommendations).
- c. If using non-Promega products to analyze the sperm fraction, a DNA purification protocol, such as the Maxwell® FSC DNA IQ™ Casework Kit (Cat.# AS1550), may be required.

7. Storing and Using Non-Sperm and Sperm Fractions in Downstream Applications

The non-sperm and sperm fractions can be stored at -30°C to $+10^{\circ}\text{C}$ for at least 3 months.

7.A. Recommendations for Using the Non-Sperm Fraction

For best results, we recommend purifying the non-sperm fraction before use in downstream processing with the Maxwell® FSC DNA IQ™ Casework Kit (Cat.# AS1550). Follow an abridged protocol for Section 3.B Extraction of Liquid Samples in the *Maxwell® FSC DNA IQ™ Casework Kit Technical Manual #TM499*. The abridged protocol begins with Step 4 (skipping Steps 1–3) and continues through the remaining steps. Use a maximum of 400µl of non-sperm fraction per purification.

If purified in this fashion, the non-sperm fraction does not require adding 5X AmpSolution™ Reagent when used with Promega short-tandem repeat (STR) chemistries or qPCR systems.

Optional: You may be able to use the non-sperm fraction in Promega short-tandem repeat (STR) chemistries, including the PowerQuant® and PowerPlex® systems, without purification if the Proteinase K is heat inactivated by placing the closed tubes containing the non-sperm fraction in a 95°C heat block or water bath for 5 minutes. **Do not allow samples to remain on the heat block for longer than 5 minutes; longer incubation times at 95°C might degrade the DNA.** After incubation, handle the tubes with care and cool the sample to room temperature before opening or vortexing to prevent potential spillage. Purification might still be needed after this heat inactivation, depending on sample type and application.

7.B Recommendations for Using the PowerQuant® System

The amount of human genomic DNA present in the non-sperm and sperm fractions can be quantified using the PowerQuant® System. Follow the protocol in the *PowerQuant® System Technical Manual #TMD047* without any changes, using 2µl of the non-sperm or sperm fraction per 20µl of PowerQuant® System amplification reaction. In addition, the human DNA quality in these extracts can be evaluated using the PowerQuant® data. The ratio of the autosomal-to-degradation target quantification results can indicate whether or not the human DNA is degraded, whereas the ratio of the autosomal-to-Y target quantification results can provide information on the ratio of female-to-male DNA in the sample. The PowerQuant® IPC target performance can indicate whether PCR inhibitors are present, which are likely to inhibit downstream STR amplification.

7.C. Recommendations for Using the Plexor® HY System

The amount of human genomic DNA in the non-sperm and sperm fractions can be quantified using the Plexor® HY System if 5X AmpSolution™ Reagent (Cat.# DM1231) is added to the DNA quantification reaction. The non-sperm and sperm fractions will inhibit qPCR amplification if 5X AmpSolution™ Reagent is not added to the reaction. Include the 5X AmpSolution™ Reagent in the DNA standard curve reactions as well as in the non-sperm and/or sperm fraction amplifications. In addition to quantification, the ratio of the autosomal-to-Y target quantification results can provide information on the ratio of female-to-male DNA in the sample, whereas the Plexor® HY IPC target can indicate whether or not PCR inhibitors are present that are likely to inhibit downstream STR amplification. Modify the Plexor® HY reaction mix described in the reaction setup section of the instrument-specific Plexor® HY System Technical Manual as described in Table 4.

Table 4. qPCR Amplification Mix for the Plexor® HY System.

Component	Volume per Reaction
Water, Amplification Grade	3µl
Plexor® HY 2X Master Mix	10µl
Plexor® HY 20X Primer/IPC Mix	1µl
5X AmpSolution™ Reagent	4µl
Total volume	18µl

Add 2µl of extract to 18µl of Plexor® HY reaction mix for a 20µl final reaction volume.

7.D. Recommendations for Using the PowerPlex® ESI Fast and ESX Fast Systems

The PowerPlex® ESI Fast and ESX Fast Systems require 5X AmpSolution™ Reagent for efficient amplification of the non-sperm and sperm fractions (see Table 5).

Table 5. PCR Amplification Mix for PowerPlex® ESI Fast and ESX Fast Systems.

PCR Amplification Mix Component	Volume Per Reaction	×	Number of Reactions	=	Final Volume
PowerPlex® ESI/ESX Fast 5X Master Mix	5.0µl	×		=	
PowerPlex® ESI 16/17 or ESX 16/17 Fast 10X Primer Pair Mix	2.5µl	×		=	
5X AmpSolution™ Reagent	2.5µl	×		=	
Total reaction mix volume	10.0µl				

Add up to 15µl of non-sperm or sperm fraction to 10µl of amplification reaction mix.

Perform thermal cycling and subsequently analyze the amplified products using capillary electrophoresis as described in the technical manuals for the PowerPlex® ESI Fast or ESX Fast System, as appropriate.

7.E. Amplifying with PowerPlex® Systems That Do Not Require 5X AmpSolution™ Reagent

Amplification with the eight-dye PowerPlex® systems (PowerPlex® 35GY and PowerPlex® 18E Systems) do not require 5X AmpSolution™ Reagent.

The PowerPlex® Fusion, PowerPlex® Fusion 6C, PowerPlex® 21 and PowerPlex® Y23 Systems may not require 5X AmpSolution™ Reagent. Set up the reaction for each PowerPlex® System in the same way according to the corresponding technical manual. PowerPlex® Fusion 6C (Table 6) and PowerPlex® Y23 Systems (Table 8) are shown as examples below.

Note: In some cases, partial inhibition may be seen with a maximal sample volume of 15µl. This is manifested by increased baseline noise and reduced heights for some larger amplicons. This can be mitigated by adding 1µl of 5X AmpSolution™ Reagent to the 25µl PowerPlex® amplification reaction and reducing the maximum sample volume to 14µl as shown in Table 7 (except for the PowerPlex® Y23 System, where including 1µl of 5X AmpSolution™ Reagent increases the maximum sample volume to 16.5µl as shown in Table 9).

Table 6. PCR Amplification Mix for PowerPlex® Fusion 6C System.

PCR Amplification Mix Component	Volume Per Reaction	×	Number of Reactions	=	Final Volume
PowerPlex® Fusion 6C 5X Master Mix	5µl	×		=	
PowerPlex® Fusion 6C 5X Primer Pair Mix	5µl	×		=	
Total reaction mix volume	10µl	×		=	

Add up to 15µl of non-sperm or sperm fraction to 10µl of amplification reaction mix. This setup will be the same for PowerPlex® 21 and PowerPlex® Fusion kits, using 10µl of amplification reaction mix.

Table 7. PCR Amplification Mix for PowerPlex® Fusion 6C System with 5X AmpSolution™ Reagent.

PCR Amplification Mix Component	Volume Per Reaction	×	Number of Reactions	=	Final Volume
PowerPlex® Fusion 6C 5X Master Mix	5µl	×		=	
PowerPlex® Fusion 6C 5X Primer Pair Mix	5µl	×			
5X AmpSolution™ Reagent	1µl	×		=	
Total reaction mix volume	11µl	×		=	

Add up to 14µl of non-sperm or sperm fraction to 11µl of amplification reaction mix. This setup will be the same for PowerPlex® 21 and PowerPlex® Fusion kits, using 11µl of amplification reaction mix.

Table 8. PCR Amplification Mix for PowerPlex® Y23 System.

PCR Amplification Mix Component	Volume Per Reaction	×	Number of Reactions	=	Final Volume
PowerPlex® Y23 5X Master Mix	5.0µl	×		=	
PowerPlex® Y23 10X Primer Pair Mix	2.5µl	×			
Water, Amplification Grade	2.5µl	×			
Total reaction mix volume	10.0µl	×		=	

Add up to 15µl of non-sperm or sperm fraction to 10µl of amplification reaction mix.

Table 9. PCR Amplification Mix for PowerPlex® Y23 System with 5X AmpSolution™ Reagent.

PCR Amplification Mix Component	Volume Per Reaction	×	Number of Reactions	=	Final Volume
PowerPlex® Y23 5X Master Mix	5.0µl	×		=	
PowerPlex® Y23 10X Primer Pair Mix	2.5µl				
5X AmpSolution™ Reagent	1.0µl	×		=	
Total reaction mix volume	8.5µl	×		=	

Add up to 16.5µl of non-sperm or sperm fraction to 8.5µl of amplification reaction mix.

Perform thermal cycling and subsequent analysis of amplified products by capillary electrophoresis as described in the technical manuals for the PowerPlex® Fusion, PowerPlex® Fusion 6C, PowerPlex® 21 or PowerPlex® Y23 System, as appropriate.

7.F. Recommendations for Using Other Quantification and STR Systems and Applications

If a non-Promega chemistry is used for human DNA quantification or STR analysis, you may need to further purify the fractions. We recommend that the sperm and non-sperm fractions be cleaned up using the Maxwell® FSC DNA IQ™ Casework Kit (Cat# AS1550), following an abridged protocol for Section 3.B Extraction of Liquid Samples in the *Maxwell® FSC DNA IQ Casework Kit Technical Manual #TM499*. The abridged protocol begins with Step 4 (skipping Steps 1–3) and continues through the remaining steps. Use a maximum of 400µl of sperm or non-sperm fraction per purification.

8. Troubleshooting

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

Symptoms	Causes and Comments
Low DNA yield in sperm fraction	<p>Yields can be influenced by sample properties, such as substrates or other materials present.</p> <p>Certain materials, such as blood or denim, may result in lower yields.</p> <p>The sperm pellet might have been lost during the preprocessing step. Do not aspirate or otherwise disrupt the pellet before resuspending in Section 4.D, Step 7.</p> <p>An old 1-Thioglycerol dilution may have been used. Ensure that all 1-Thioglycerol dilutions are freshly made for each use.</p>
Low DNA yield in non-sperm fraction or highly degraded DNA in the non-sperm fraction	<p>If the non-sperm fraction was heat-inactivated following the optional protocol, the samples may have been heated for too long. Do not heat non-sperm fractions at 95°C for longer than 5 minutes.</p>
Low DNA yield for the non-sperm or sperm fraction when using the Maxwell® FSC DNA IQ™ Casework Kit (Cat.# AS1550)	<p>If the non-sperm fraction was heat-inactivated following the optional protocol, the samples may have been heated for too long. Do not heat non-sperm fractions at 95°C for longer than 5 minutes.</p> <p>If the sperm fraction was heat inactivated before using the Maxwell® FSC DNA IQ™ Casework Kit, the heat inactivation may have been performed at an excessively high temperature. Ensure that sperm fractions are heat inactivated at 70°C.</p>
PCR inhibition in sperm or non-sperm fraction	<p>The fraction may contain a sample-derived PCR inhibitor. Purify the DNA using a kit such as the Maxwell® FSC DNA IQ™ Casework Kit (Cat.# AS1550).</p> <p>Fractions are compatible with Promega PCR chemistries. If you are using a different PCR chemistry, you may need to purify DNA first (see Section 7.F).</p> <p>Certain Promega chemistries will require the use of 5X AmpSolution™ Reagent. See Section 7.</p> <p>The fraction may not have been appropriately heat inactivated. Ensure that sperm fractions were heat inactivated (Section 6, Step 13) and that non-sperm fraction was either purified or heat-inactivated (Section 7.A).</p>

Symptoms

Causes and Comments

Elution tubes are empty following a run	<p>An insufficient volume of elution mix might have been added to the elution tubes. Some amount of evaporation will occur during the elution step Maxwell Differential Extraction method.</p> <p>The tubes were left open on the Maxwell® deck too long after completion of the run, allowing time for additional evaporation.</p> <p>An incompatible plunger was used. Make sure to only use plungers supplied with the kit.</p>
Instrument errors	<p>Verify that nothing is physically blocking the movement of the platform, plunger bar or magnetic rod assembly.</p> <p>Perform a Self Test from the Settings menu. The instrument will rehome itself. If the error recurs, contact Promega for service.</p> <p>The cartridges were not completely seated on the platform. Ensure the cartridges are pressed firmly into place.</p> <p>Incorrect elution tube was used. Use only the Elution Tube, 0.5ml, provided with the Maxwell® DE System. Other tubes may have different dimensions.</p>
Resin carryover during elution	<p>The presence of a small amount of resin particles in the Elution Tube will not affect the final DNA concentration or downstream applications. If desired, final elution tubes can be centrifuged to pellet resin and eluates can be transferred to a clean tube or an additional resin capture step may be performed using the 0.5ml MagneSphere® Technology Magnetic Separation Stand (Cat.# Z5341).</p>
Plungers on rod after a run	<p>Consult the appropriate Maxwell® instrument operating manual (see Table 3, Section 5) and perform the indicated Clean Up protocols.</p>

9. Related Products

Product	Size	Cat.#
Maxwell® FSC Instrument	each	AS4600
Maxwell® RSC Instrument	each	AS4500
Maxwell® RSC 48 Instrument	each	AS8500
ClickFit Microtube, 1.5ml	100/pack	V4745
DNA IQ™ Spin Baskets	50/pack	V1225
Elution Tubes, 0.5ml	50/pack	AS7201

10. Summary of Changes

The following changes were made to the 9/25 revision of this document:

1. Updated Proteinase K quantity in Section 2 and volume in Section 4.B, Table 1.
2. Added new notes in Sections 4.A, 4.C and 5.
3. Updated Sections 3, 4.D and 8.
4. Revised heat block temperature in Section 6, Step 13.
5. Added new Section 7.A and renumbered subsequent sections.

^(a)Patent Pending.

© 2024–2025 Promega Corporation. All Rights Reserved.

MagneSphere, Maxwell, Plexor and PowerPlex are registered trademarks of Promega Corporation. AmpSolution and DNA IQ are trademarks of Promega Corporation.

Products may be covered by pending or issued patents or may have certain limitations. Please visit our website for more information.

All prices and specifications are subject to change without prior notice.

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.