

Promega

MaxSpin[™] 96 Preprocessing of the Maxwell[®] FSC DNA IQ[™] Casework Kit for the Maxprep[™] Liquid Handler

Instructions for Use of Products AS9100, AS9101, AS9200 and AS9201

Use in combination with the Preprocessing Methods for the Maxprep[™] Liquid Handler Technical Manual #TM529.

MaxSpin[™] 96 Preprocessing of the Maxwell[®] FSC DNA IQ[™] Casework Kit for the Maxprep[™] Liquid Handler

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

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1. Description

The MaxSpin[™] 96 Casework preprocessing protocol for the Maxprep[™] Liquid Handler is divided into two Maxprep[™] methods.

- The MaxSpin[™] 96 Casework Extraction method extracts casework solid-support samples in the MaxSpin[™] 96.
- The Maxwell[®] FSC DNA IQ[™] MaxSpin[™] 96 method prepares Maxwell[®] deck trays for the Maxwell[®] FSC DNA IQ[™] Kit using extracted samples in the MaxSpin[™] 96 plate.

The Maxprep[™] Liquid Handler can perform the heated extraction of the solid-support samples and preprocess the extracts into Maxwell[®] FSC Cartridges. Administrators can create variant methods in the Maxprep[™] software that specify preprocessing options to meet the needs of the laboratory. Creating variant preprocessing methods is described in the Maxprep[™] Liquid Handler Technical Manual #TM529. More information about casework extraction is described in the Maxwell[®] FSC DNA IQ[™] Casework Kit Technical Manual #TM499; casework direct protocol is described in the Casework Direct System Technical Manual #TMD067.



2. Materials to Be Supplied by the User

- Maxwell[®] FSC DNA IQ[™] Casework Kit (Cat.# AS1550)
- Casework Extraction Kit (Cat.# DC6745)
- Casework Direct System (Cat.# DC4560, DC4561)
- MaxSpin[™] 96 Kit (Cat.# AS9040)
- Maxprep[™] 1,000µl Conductive Disposable Tips, Filtered (Cat.# AS9303)
- Maxprep[™] 300µl Conductive Disposable Tips, Filtered (Cat.# AS9302)
- Maxprep[™] Reagent Reservoir, 50ml (Cat.# AS9304)
- Maxprep[™] Plunger Holder (Cat.# AS9408)

3. MaxSpin[™] 96 Setup with Casework Samples on a Solid Support

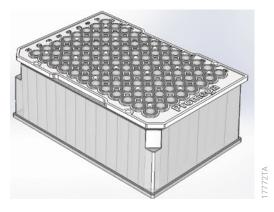


Figure 1. The assembled MaxSpin[™] 96 in the sample lysis configuration.

- 1. To begin, set aside the collar until ready for centrifugation, and align the frame with the plate.
- 2. Each solid-support sample can be placed into a basket before being placed into a well of the MaxSpin[™] 96. For swabs, place head-down into the basket. The swab should be cut or snapped off the swab handle close to the head and, if needed, the remaining stick can be used to push the head to the bottom of the basket.
- 3. Using Portal Access, a plate map of sample bar codes can be entered as the dry samples are being placed into the MaxSpin[™] 96. Scanning the bar code of the MaxSpin[™] 96 will automatically pull the associated sample bar codes from Portal into the Casework Extraction, Cartridge Preparation or both methods.
- 4. Once the MaxSpin[™] 96 is full or all the samples are placed, proceed to the MaxSpin[™] 96 Casework Extraction method.



4. MaxSpin[™] 96 Sample Extraction

The MaxSpin[™] 96 Casework Extraction method can run either Casework Extraction or Casework Direct protocols with the incubation either on-deck or on a separate heat block, depending on variant settings. The default method runs Casework Extraction with an on-deck incubation.

- In the Maxprep[™] software, touch Start to access the 'Methods' screen. On the 'Methods' screen, touch the MaxSpin[™] 96 Casework Extraction method or laboratory-specific variant of the MaxSpin[™] 96 Casework Extraction method.
- 2. Verify that the appropriate preprocessing method or variant method has been selected and touch the **Proceed** button. Touch the **Run** button on the method run screen to start the run.
- The first screen of the method gathers information about the MaxSpin[™] 96. Scan or enter the unique identifier of the MaxSpin[™] 96 and select the plate icon to verify or manually enter the location and IDs of the samples in the MaxSpin[™] 96.
- Uncheck the "On Deck Incubation" check box if the plate will be incubated on a different device.
 Note: Do not shake the MaxSpin[™] 96 during incubation. Keep the MaxSpin[™] 96 still and uncovered.
- 5. Follow instrument setup instructions displayed in the method. The Maxprep[™] software will direct you where to place the MaxSpin[™] 96.

te 1: Extraction Mix	Site 1: Casework Direct Solution
Combine the following:	Combine the following:
Reagent Volume Lot Number Casework Extraction Buffer 40 mL Proteinase K 1035 µL	Reagent Volume Lot Number Casework Direct Reagent 41 mL Diluted 1-Thioglycerol 207 µL
Mix the solution, pour into a Trough, place on deck.	Mix the solution, pour into a Trough, place on deck.

Figure 2. Reagent detail popup for the Extraction Mix (Panel A) or Casework Direct Solution (Panel B) methods. The details window will specify the volume of Casework Extraction Buffer, Proteinase K, 1-Thioglycerol or Casework Direct Reagent and Diluted 1-Thioglycerol (in ml or µl) to combine and place in a trough, depending on which extraction method is chosen in the method variant.



4. MaxSpin[™] 96 Sample Extraction (continued)

6. Reagent setup instructions include where to place the Maxprep[™] 50ml Reagent Reservoir filled with Extraction Mix. Select the **Enter Reagent Details** button to determine what volumes of Casework Extraction Buffer, Proteinase K, 1-Thioglycerol or Casework Direct Reagent and Diluted 1-Thioglycerol to add together. Invert to mix the components in a tube before placing into the reagent trough.

Note: The 1-Thioglycerol used in Casework Direct should be diluted tenfold in Water, Amplification Grade. For example, add 20µl of 1-Thioglycerol to 180µl of Water, Amplification Grade. Diluted 1-Thioglycerol is stable at 4°C for 6 months.

- 7. Add Maxprep[™] 1,000µl Conductive Disposable Tips, Filtered (2; one rack may be partially full).
- 8. Close the instrument door and touch the Next button to start the automated sample extraction.
- 9. If on-deck heating is deselected, once the method is completed, remove the MaxSpin[™] 96 from the Maxprep[™] deck and place on a compatible heating device for the heated incubation.

Note: Do not shake the MaxSpin[™] 96 during incubation. Keep the MaxSpin[™] 96 still and uncovered.

5. MaxSpin[™] 96 Centrifugation

1. After incubation, add the MaxSpin[™] 96 collar under the frame. Do not lift the baskets completely out of the wells, but instead lift the frame just enough to slide the collar into place. Match the alignment features on the collar with the holes in the frame to properly seat the collar.

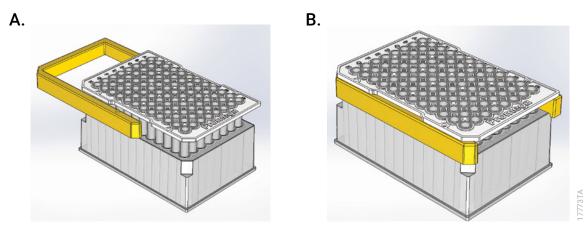


Figure 3. Adding the MaxSpin[™] 96 collar prepares the MaxSpin[™] 96 for centrifugation. Panel A. After the heated incubation, lift the frame just high enough to slide the collar into place. Do not lift the baskets completely out of the plate. Panel B. When the collar is aligned with the holes in the frame, the MaxSpin[™] 96 is ready to be centrifuged.

- 2. Centrifuge the MaxSpin^M 96 for 3 minutes at \geq 500 × g.
- 3. Discard the collar, frame, baskets and dried solid supports.

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6. Maxwell® FSC DNA IQ[™] Casework Cartridge Preparation from a MaxSpin[™] 96

Prior to running this method, samples must be prepared by the MaxSpin[™] 96 Casework Extraction method. MaxSpin[™] 96 plates should have the extracts centrifuged through and the dry solid supports removed.

- 1. In the Maxprep[™] software, touch **Start** to access the 'Methods' screen. On the 'Methods' screen, select a method using one of the two options below:
 - a. Touch the Maxwell[®] FSC DNA IQ[™]-MaxSpin[™] 96 method.
 - b. Use a bar code reader to scan the 2D bar code on the kit box to filter the available methods for the Maxwell[®] FSC DNA IQ[™] Casework Kit. Touch the Maxwell[®] FSC DNA IQ[™] –MaxSpin[™] 96 method.
- 2. Verify that the appropriate preprocessing method or variant method has been selected and touch the **Proceed** button. Touch the **Run** button on the method run screen to start the run.
- 3. Enter any method-specific variables (Elution Volume, Maxwell Type) on the initial method screen.
- 4. Scan or enter the unique identifier of the MaxSpin[™] 96 plate. Optionally, select the "Process Previously Run Samples" check box to see and process samples that have already been run through the method. Choose **Exit**.
- 5. Optionally, select the **Add Sample or Remove Sample** button to manually change which samples should be processed. Select the **Requery Portal** button to reimport the MaxSpin[™] 96 plate layout from Portal or to process a different MaxSpin[™] 96 plate.
- 6. Prior to placing Maxwell[®] deck tray(s) on the Maxprep[™] Liquid Handler, prepare the deck tray(s) with cartridges and elution tubes. Change gloves before handling Maxwell[®] FSC Cartridges and Elution Tubes (0.5ml). Place the cartridges to be used in the deck tray(s) with well #1 (the largest well in the cartridge) facing away from the elution tubes. Press down on the cartridge to snap it into position. Carefully peel back the seal so that all plastic comes off the top of the cartridge. Ensure that all sealing tape and any residual adhesive are removed before placing cartridges in the instrument. Place an open, empty elution tube into the elution tube position for each cartridge in the deck tray(s). The elution tube cap should open outwards and away from the cartridges.

Notes:

- 1. Sample 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.
- 2. Use only the 0.5ml Elution Tubes provided in the kit; other tubes may be incompatible with the Maxwell[®] Instrument.



6. Maxwell[®] FSC DNA IQ[™] Casework Cartridge Preparation from a MaxSpin[™] 96 (continued)

- 7. Follow instrument setup instructions displayed in the method. You will be directed by the Maxprep[™] software where to place the following items on the instrument:
 - Maxprep[™] Plunger Holders and Maxwell[®] FSC Plunger Packs (2; one may be partially full)
 - 24-position Maxwell[®] Front Deck Tray or 16-position Maxwell[®] Deck Tray containing Maxwell[®] FSC Cartridges with seals removed and open elution tubes
 - 24-position Maxwell[®] Back Deck Tray or 16-position Maxwell[®] Deck Tray containing Maxwell[®] FSC Cartridges with seals removed and open elution tubes
 - Maxprep[™] Reagent Reservoir, 50ml, with Elution Buffer
 - Maxprep[™] Reagent Reservoir, 50ml, with Lysis Buffer
 - Centrifuged MaxSpin[™] 96 Plate (baskets removed)
 - Maxprep[™] 1,000µl Conductive Disposable Tips, Filtered (2; one rack may be partially full)
 - Maxprep[™] 300µl Conductive Disposable Tips, Filtered (rack may be partial or full)
- 8. Close the instrument door and touch the **Next** button to start the automated preprocessing of samples.
- 9. The Maxprep[™] Liquid Handler will prepare samples prior to extraction using a Maxwell[®] Instrument. The Maxprep[™] Liquid Handler performs the following steps:
 - a. Plungers are transferred to each of the cartridges in the Maxwell[®] deck tray(s). The specified volume of Elution Buffer is transferred to the elution tubes for each position in the Maxwell[®] deck tray(s).
 - b. Lysis buffer is added to each sample then the lysed sample is transferred from the MaxSpin[™] 96 plate to its corresponding Maxwell[®] FSC Cartridge.

When the method is complete, open the instrument door and move the deck tray(s) to the Maxwell[®] Instrument for extraction. Remove the MaxSpin[™] 96 plate and used tips from the waste bin of the instrument and discard as hazardous waste following your institution's recommended guidelines. Either discard or tightly cap and store remaining reagents.

Consumables for Maxprep[™] preprocessing methods are designed to be used with potentially infectious substances. Use appropriate protective equipment (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.

For information on running the deck trays on a Maxwell[®] Instrument, see the Maxwell[®] FSC DNA IQ^{m} Casework Kit Technical Manual #TM499.

7. Variant Method Variables

Administrators should create laboratory-specific variants of the MaxSpin[™] 96 Casework preprocessing methods for each unique preprocessing and Maxwell[®] setup they wish to create. The following sections describe the variables that can be adjusted by administrators. By default, users can modify some these variables at run time; toggle the "User Modifiable" check box to allow or prevent user access at run time. The administrator has the ability to define the default value for the variable within the displayed minimum and maximum allowable values.

7.A. Variant Variables for MaxSpin[™] 96 Casework Extraction

Setting	Details
Heated Incubation on Deck?	Checking this box will run the MaxSpin [™] 96 heated incubation on the HHS heater on the Maxprep. Unchecking will cause the method to prompt the user to incubate the plate on a different device.
Incubation Temperature (°C)	This variable defines the target incubation temperature, in Celsius, of the heated incubation. A correction to the incubation temperature is automatically applied to the heater module. Additional correction should not be required.
Incubation Time (min)	This variable defines the duration of the incubation, in minutes, starting once the heater reaches temperature.
Casework Direct Reagent Volume (µl)	The volume of Casework Direct Reagent to be added to each sample. The total volume of Casework Direct Reagent + Diluted 1-Thioglycerol must equal to a total volume between 100 and 400, and the total volume must also be evenly divisible by 50.
Diluted 1-Thioglycerol Volume (µI)	The volume of prediluted 1-Thioglycerol to be added, per sample. The 1-Thioglycerol should be diluted tenfold in Water, Amplification Grade. Used for generating the user prompt.
Use Casework Direct?	Checking this box will run the Casework Direct extraction. The method will switch to using the volumes set for Casework Direct Reagent and Diluted 1-Thioglycerol. If unchecked, those two volumes are ignored and the volumes for the Casework Extraction reagents are used. If this is checked, we recommend changing the incubation temperature to 70°C.
1-Thioglycerol Volume (µI)	The volume of 1-Thioglycerol to be added, per sample. Used for generating the user prompt.

These variables define how a method should extract samples.



Setting	Details
Casework Extraction Buffer Volume (µI)	The volume of Casework Extraction Buffer to be added, per sample. The total volume of Casework Extraction Buffer + 1-Thiolgycerol + Proteinase K must equal a total volume between 100 and 400. The total volume must also be evenly divisible by 50.
Proteinase K Volume (μΙ)	The volume of Proteinase K to be added, per sample. Used for generating the user prompt.

7.B. Variant Variables for Maxwell® FSC DNA IQ[™]-MaxSpin[™] 96

These variables define how a method should process samples for the cartridge setup.

Setting	Details
Limit to Single Deck Tray?	Checking this box will limit the maximum sample number to what can fit on one deck tray.
Maxwell FSC (16 Sample) Instrument	This check box defines the default deck tray that will be selected during the preprocessing method setup.
Elution Volume (µI)	This variable defines the default volume that will be dispensed into the Elution Tube.
Sample Volume (µl)	This variable defines the volume of sample. It should match the total volume of the extraction mix.

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