**TNA (RNA), Tissue Automated Extraction Procedure**

1. **PRINCIPLE:**
	1. Isolation of TNA from tissue samples is critical for the success of several assays in the Molecular Genomic Pathology Laboratory. The Maxwell RSC RNA FFPE Kit excluding the provided DNases is used in combination with the Maxwell RSC Instrument to provide an easy method for efficient, automated purification of TNA from formalin-fixed, paraffin-embedded FFPE tissue samples.
	2. The Maxwell RSC Instrument is a magnetic particle-handler that allows efficient binding of TNA to the paramagnetic particles in the first well of the prefilled cartridge and moves the samples through the cartridge wells, mixing during processing.
	3. The quality of TNA produced is dependent on the starting material, as well as the method of preparation. TNA of quality satisfactory for PCR can usually be obtained from standard formalin-fixed, paraffin-embedded (FFPE) tissue block samples.
	4. To aid in purification of TNA from tissue, samples are digested with the active protease, Proteinase K, in the presence of ionic detergent and the chelating agent EDTA. This protocol rapidly and irreversibly inhibits naturally occurring DNases in each sample and results in TNA of sufficient quality for all assays performed in the laboratory. This digestion step also results in the rapid inactivation of conventional infectious agents. This method adapts well to the isolation of TNA from FFPE blocks, following removal of paraffin by treatment with xylene and ethanol.
2. **SAMPLES:**
	1. Sample types: Formalin-fixed, paraffin embedded (FFPE) tissue (slides or blocks)
3. **REAGENTS:**
	1. Promega Maxwell RSC RNA FFPE kit: Cat # AS1440. Stored at room temperature.
4. **PROCEDURE FOR RNA, Tissue Automated Extraction:**
	1. For specimens that exhibit heavy melanin pigmentation, consult Lab Director to determine if the standard protocol or the BSA Method should be utilized. Refer to the PCR Interfering substances BSA Method Procedure.
		1. If BSA is necessary, add an internal note in Soft Molecular.
	2. **RSC Cut Block/RSC Scrape Slides Action**
		1. Log into Soft Molecular.
		2. Open Extractions by using the Extractions tile on the dashboard.
		3. Highlight RSC Cut Block or RSC Scrape Slides branch in the action tree.
		4. Highlight the Barcode# field. Scan the Soft Molecular specimen label and press **Enter** on the keyboard. Repeat this step for all applicable specimens.
		5. **If a sample is being re-extracted, expand the child level, and change the Volume in the child level** **tube to 200. This is necessary to ensure adequate volume in the system for performing any testing.**
		6. Select **Save**.
			1. Two aliquot labels per sample will automatically print upon saving.
				1. If only one 1.5ml tube is being utilized, discard the second aliquot label in the appropriate receptacle.
		7. Select **Back** in the Extractions window.
		8. Exit Soft Molecular application.
		9. Label a 1.5ml tube with an aliquot label for each specimen.
	3. **Record your extraction reagents: RSC Extraction Reagents**
		1. Highlight the RSC Extraction Reagents branch on the action tree.
		2. Highlight the Barcode# field. Scan the aliquot label and press **Enter** on the keyboard. Repeat this step for all applicable specimens.
		3. Enter the final elution volume in the Prod Vol column.
			* 1. FFPE Block/Unstained Slides: 35ul
		4. Select the Spec/Tube Reagents field and in the dropdown, scan the appropriate TQC reagent label to add the RSC Maxwell RNA FFPE kit lot number to each specimen.
		5. Select **OK** in the Spec/Tube Reagent window.
		6. Mark the **Completed** checkbox and select **Save**.
	4. **Cut Block/Scrape Slides**
		1. Cut FFPE blocks and macrodissect unstained slides according to *FFPE Tissue Preparation for Molecular Testing* Procedure.
		2. For FFPE blocks, use a microtome set at 5 microns and cut 5 slices/scrolls per sample.
		3. For macrodissection of tissue on unstained slides, use 8 slides per sample or number of slides otherwise noted.
		4. Spin tissue to the bottom of the tube in microcentrifuge.
		5. Prepare a Blank reaction tube for each Maxwell® RSC run for quality control.
			* 1. Label a 1.5mL microcentrifuge tube as the “Blank”.
				2. Process the Blank reaction along with the samples.
	5. **Preprocessing of FFPE Samples**
		1. Add 300µL of Mineral Oil to each sample and Blank tubes.
		2. Vortex for 10 seconds.
		3. Ensure that the tissue section is completely immersed in the Mineral Oil.
		4. Heat the samples at 80°C for 2 minutes.
		5. After the 2 minutes of incubation, place the samples at room temperature while the master mix is prepared.
			1. **NOTE**: Use the master mix within 1 hour of preparation. The master mix cannot be stored for later use.
		6. Prepare a master mix of Lysis Buffer, Proteinase K Solution and Blue Dye as shown below.
		7. See Table 1 for Master Mix preparation:

**Table 1: The RSC Master Mix Preparation**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Number of Samples/****Reaction Tubes** | **Volume of Lysis Buffer****(µL)** | **Volume of Proteinase K (µL)** | **Volume of Blue Dye****(µL)** | **Total Volume** **(µL)** |
| 1 | 448 | 50 | 2 | 500 |
| 2 | 672 | 75 | 3 | 750 |
| 3 | 896 | 100 | 4 | 1000 |
| 4 | 1120 | 125 | 5 | 1250 |
| 5 | 1344 | 150 | 6 | 1500 |
| 6 | 1792 | 200 | 8 | 2000 |
| 7 | 2016 | 225 | 9 | 2250 |
| 8 | 2240 | 250 | 10 | 2500 |
| 9 | 2464 | 275 | 11 | 2750 |
| 10 | 2688 | 300 | 12 | 3000 |
| 11 | 2912 | 325 | 13 | 3250 |
| 12 | 3136 | 350 | 14 | 3500 |
| 13 | 3360 | 375 | 15 | 3750 |
| 14 | 3584 | 400 | 16 | 4000 |
| 15 | 3808 | 425 | 17 | 4250 |
| 16 | 4032 | 450 | 18 | 4500 |

* + 1. Vortex the master mix for 5 seconds to mix.
		2. Add 250µL of master mix to each reaction tube.
		3. Vortex each tube for 5 seconds.
		4. Centrifuge the tubes at 10,000 x g for 30 seconds to separate the layers.
			1. **NOTE**: If a large pellet remains in the lower blue layer, mix the lower blue layer with a 1mL pipette tip to resuspend the pellet. Centrifuge the tube again at 10,000 x g for 30 seconds to separate the layers.
		5. Transfer the tubes to a 56°C heat block.
		6. Incubate the samples at 56°C for 15 minutes.
		7. After the 56°C incubation, immediately transfer the samples to an 80°C heat block.
		8. Incubate the samples at 80°C for 1 hour.
		9. After the 80°C incubation, remove the samples from the heat block.
		10. Allow the samples to cool to room temperature for 8 minutes.
		11. After the samples reach room temperature, add 50µL of Nuclease-Free Water to the lower blue layer in each tube. Then, pipette 5 times to mix.
		12. Centrifuge the samples at full speed in a microcentrifuge for 2 minutes.
		13. The samples are now preprocessed and ready to be loaded onto the Maxwell® RSC for purification.
	1. **Initialize the Maxwell® RSC System:**
		1. If the Maxwell® RSC Instrument and the Tablet PC are already powered on, proceed to Step 10 below; otherwise, continue to Step 2.
		2. Start the System by powering on the Maxwell® RSC and the Tablet PC.
		3. The RSC power switch is located next to the power cable connection on the back-left side of the instrument.
		4. Power on the instrument by switching the On/Off power switch to “On”.
		5. Press the power button located on the top of the Tablet PC to power on the Tablet.
		6. Launch the Maxwell® RSC Application Software by touching the software icon on the touchscreen.
		7. A Self-Test is performed automatically when the instrument is powered on and the software launched.
		8. The Self-Test checks the Maxwell® RSC’s initialization, motion, and heater functions.
		9. The Self-Test can also be manually initiated if the System is already on.
		10. From the Home Screen, select the Settings tab and then, select Self-Test to perform the Self-Test.
			1. **NOTE**: A Self-Test must be performed and passed before each use of the instrument.
	2. **Setup Maxwell RSC Instrument:**
		1. From the Home Screen, select **Start**.
		2. Touch the text box at the top of the screen to enter the kit barcode ID.
		3. Using the keypad, enter the entire barcode ID, which can be found on the label of the kit or use a scanner to scan the barcode (e.g., AS14401041092020-08).
			1. **NOTE**: The kit barcode ID is a combination of the catalog ID, lot number, and the expiration date of the kit and must be valid to run a Method.
		4. When the barcode ID is entered correctly, the corresponding extraction Method from the list of preprogrammed Methods will be highlighted.
		5. Confirm that the highlighted Method matches the extraction Kit being used.
		6. Touch the **Proceed** buttonnext to the highlighted Method, to move to the Cartridge Setup screen.
		7. The Cartridge Setup screen displays the layout of the Maxwell RSC Deck Tray with position numbers and the selected run Method.
		8. On the Cartridge Setup screen, the user can select which cartridge positions (based on the number of samples) will be processed by selecting or deselecting cartridge positions.
		9. Select a cartridge position by touching the black rectangle above the number of that position.
		10. A selected position can be deselected/deactivated by touching the black rectangle of that position again.
			1. See the Maxwell® RSC Cartridge Setup Screen in Figure 1 and the Cartridge Setup Screen with Sample ID input in Figure 2:

**Figure 1: Cartridge Setup screen**



**Figure 2: Sample ID input screen**



* + 1. **NOTE:** The instrument will only process cartridge positions that have been selected. Therefore, ensure that no cartridges are loaded in positions that have been excluded from processing.
		2. Next, enter the corresponding Sample ID and Elution Tube ID for all cartridge positions that are to be processed.
		3. To enter the Sample ID and Elution Tube ID for a position, touch the “active” position number (black square) on the Cartridge Setup screen.
		4. Touch the **Sample ID** text box and use the keypad to enter the Sample ID or use a scanner to scan the aliquot label (e.g., MOL-00-0000).
		5. Touch the **Elution Tube** **ID** text box and use the keypad to enter the corresponding Elution Tube position number.
		6. When all Sample IDs and Elution Tube IDs have been entered, the **Proceed** button on the bottom of the screen will become active.
		7. Touch the **Proceed** button to move to the Door screen and then press the OK button to open the Maxwell RSC Instrument door.
		8. When the Door opens, the Extraction Checklist screen is presented. The checklist indicates the steps that need to be performed prior to starting an extraction process.
		9. Follow the checklist and ensure that the Deck Tray and instrument are set up accordingly.
		10. Remove the Deck Tray from the Deck for easy loading access. Lift the tray up from the front and pull out.
			1. **NOTE:** If processing fewer than 16 samples, center the cartridges on the Deck Tray as best as possible. If helpful, label the cartridges with the Sample IDs.
		11. Clean the Maxwell® RSC instrument and deck tray before the run using a KimWipe wet with 70% ethanol.
		12. Wrap a Kimwipe wet with 70% ethanol around a magnet and wipe around the plunger bars to pick up any stray magnetic beads.
		13. Place the cartridges in the deck tray according to the previously programmed placement with well #1 (the largest well in the cartridge) facing away from the elution tubes. Press down both front and back of the cartridge to snap it into position.
			1. See the Maxwell® RSC Deck Tray with cartridges in Figure 3:

**Figure 3: Placing the Cartridges in the Deck Tray**

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* + 1. Carefully peel back the seal, making sure all the plastic comes off. If necessary, use a sterile pipette tip to help remove any remaining plastic.
		2. Verify the cartridges are still firmly snapped into place.
		3. Add each preprocessed Sample to well # 1 of the corresponding cartridges (well # 1 is the largest well in the cartridge).
			1. **NOTE**: For some difficult sample types (e.g., large pellet, abundant paraffin, etc.), the transfer process may require more than one attempt to completely transfer the blue, aqueous layer.
			2. **NOTE**: To prevent additional mineral oil and paraffin from getting into the Sample well, take care not to submerge the pipette tip below the surface of the buffer when transferring.
		4. Set a pipette to 350mL and carefully remove the entire blue, aqueous phase of the Sample.
		5. Transfer the Sample to well # 1 of the corresponding cartridge.
		6. Place one plunger into well # 8 of each cartridge (well # 8 is the closest well to the Deck Tray position numbers).
		7. Label the appropriate number of 0.2mL Elution Tubes with the corresponding Elution Tube position number found on the Tray.
		8. Add 50µL of Nuclease-Free Water to the bottom of each Elution Tube and place the Elution Tubes into the corresponding Elution Tube positions for each cartridge on the Deck Tray.
		9. Open the Elution Tubes so that the lids are oriented away from the cartridges.
			1. **NOTE:** The Elution Tubes must stay open during the purification Method.
		10. Place the prepared Tray back onto the RSC Deck by first sliding in the back end of the Tray and then pressing down on the front.
		11. When the Deck Tray has been properly seated, touch the **Start** button to begin the run.
			1. **NOTE:** Select the **Cancel** button to return to the Cartridge Setup screen.
		12. During the run, the Protocol Selected screen is shown with run information like estimated time remaining, a description of the current step being performed, and a progress bar showing the percent completion of the current step.
			1. **NOTE:** If you wish to abort the run, touch the **Abort** button in the lower right corner of the screen. Any samples being processed will be lost if a run is aborted.
			2. See Figure 4 for Placing the Deck Tray in the instrument:

**Figure 4: Placing the Deck Tray in the instrument**

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* 1. **Maxwell RSC TNA Extraction**
		1. If necessary, login to Soft Molecular and open the Extraction tile.
		2. Highlight the Maxwell TNA Extraction branch on the action tree.
		3. Highlight the Barcode# field. Scan the aliquot label and press **Enter** on the keyboard. Repeat this step for all applicable specimens.
		4. Navigate to the **Print** tab and click the **Print Product Labels** dropdown menu. Select the ‘**…**’ button that appears.
		5. Select **OK** when asked to save before print.
		6. In the Print Product Labels window, verify the correct printer and product label template is selected (PROD LBL V1). Click **Print**.
		7. Select **Back** in the Extractions window.
		8. Exit Soft Molecular application.
		9. Label a new clean 1.5ml tube with a product label for each specimen.
	2. **Completing a run:**
		1. **NOTE:** It is important to close the Sample Elution Tubes’ caps as soon as the run is finished.
		2. When the run is finished, the Protocol Selected screen will change to indicate that the protocol is 100% *Completed*.
		3. Touch the **Open-Door** button to open the door of the Maxwell® RSC Instrument.
		4. Once the Maxwell RSC door is open, verify the plungers are located in well #8.
			1. If the plungers are not located in well #8, please refer to the *Maxwell RSC Instrument Procedure* for troubleshooting steps.
			2. After the door is opened, the UV sanitization window will be presented.
		5. Immediately cap the Sample Elution Tubes to prevent evaporation and/or contamination of the eluates.
		6. Remove the Deck Tray from the instrument.
		7. Remove sample elution tubes and flash spin to collect eluate at the bottom of the tube.
		8. Place elution tubes in magnetic rack to bring any residual magnetic particles towards the center of the magnetic rack.
		9. Place empty deck tray back into Maxwell® RSC, clean with 70% ethanol and turn on UV light by selecting the sanitize icon on the tablet.
		10. Aspirate each purified sample and place into a clean and appropriately labeled 1.5mL tube.
		11. After quantification, place samples on ice for immediate use or at -80°C for storage.
	3. **Shutting down the System:**
		1. **NOTE**: Shut down the System as needed (e.g., Technical Support, or prolonged period without use).
		2. Shut down the Maxwell RSC software by pressing the ‘x’ in the upper left corner of the Home screen.
		3. Switch the Maxwell RSC “OFF” using the On/Off power switch in the back of the instrument.
		4. To shut down the Tablet PC:
			+ 1. Swipe inward from the right side of the Home screen.
				2. Select Settings.
				3. Select Power.
				4. Select Shut Down.
	4. **Refer to the Qubit 3.0 Fluorometer Instrument Procedure to measure the nucleic acid concentration of each sample.**
		1. Soft Molecular will automatically calculate dilutions to 100 ng/ul.
		2. If the sample has a concentration of greater than 150 ng/ul, then dilute the sample to 100 ng/ul.
		3. If the sample has a concentration of less than 150 ng/ul, proceed with the appropriate clinical assay.
		4. If a sample has a concentration that is too low to quantitate, in the Excel File, change “Out of range” to “0” (zero) and save as a new file with suffix “\_edit”.
	5. **DNA/TNA Quantitation Action**
		1. If your assay requires a DNA Quantitation, please refer to the DNA, Tissue Automated Extraction Procedure.
	6. **RNA Qubit Quantitation Action**
		1. Log into Soft Molecular.
		2. Open Extractions by using the Extraction tile on the dashboard.
		3. Highlight the RNA Qubit Quantitation branch on the action tree.
		4. Select **Tools** tab followed by **Import** to import Nanodrop results.
		5. Select **RNA QUBIT CONC** from the dropdown in the Template field.
		6. Choose file name using the ‘**…**’ button next to the File Name field and confirm by clicking **Open**.
		7. Select **Import**.
		8. In the Import Finished window, select **OK**.
		9. Close the Import from Excel window using the ‘**X**’.
		10. If a dilution is not required, highlight the Barcode# field, scan the product label, and select **Enter** on the keyboard. Repeat this step for all applicable specimens. Select **Save**.
		11. Select **Back** in the Extractions window.
		12. Exit Soft Molecular application.
	7. **Dilution**
		1. Mark the **Dilute(?)** checkbox for every patient sample that requires a dilution.
		2. Highlight the Barcode# field. Scan the product label and select **Enter** on the keyboard. Repeat this step for all samples pending RNA Qubit Quantitation. Select **Save**.
		3. Highlight the RNA Qubit Dilute and Repeat Quantitation branch on the action tree.
		4. Select **Tools** tab, followed by **Import** to import Qubit results.
		5. Choose file location using the ‘**…**’ button next to the Directory field and confirm by clicking **OK**.
		6. Select **FINAL RNA QUBIT CONC** from the dropdown in the Template field.
		7. Choose file name using the ‘**…**’ button next to the File Name field and confirm by clicking **Open**.
		8. Select **Import**.
		9. In the Import finished window, select **OK**.
		10. Close the Import from Excel window using the ‘**X**’.
		11. Highlight the Barcode# field, scan the product label, and select **Enter** on the keyboard. Repeat this step for all applicable specimens. Select **Save**.
		12. Select **Back** in the Extractions window.
		13. Exit Soft Molecular application.
1. **TROUBLESHOOTING:**
	1. If the Maxwell® RSC becomes contaminated:
		1. Use a paper towel wet with 10% bleach to wipe down the inside of the instrument.
		2. Follow the bleach with distilled water, and finish with 70% ethanol.
	2. For additional troubleshooting, see: Genomic DNA Clean and Concentrate Procedure.
	3. Contact information:
		1. Promega 1-800-356-9526
		2. [www.promega.com](http://www.promega.com)
		3. techserv@promega.com
2. **REFERENCES:**
	1. Maxwell® RSC Instrument Operating Manual (Revised 10/16).
	2. Maxwell® RSC RNA FFPE Kit Technical Manual (Revised 11/17).
	3. Maxwell RSC RNA FFPE Kit *Quick* Protocol with Archer Modifications.
3. **REVISIONS:**
	1. 5/11/2022: Removed Maxwell RSC Instrument steps to create a Maxwell RSC Instrument Procedure.