

PureSpeed™ Tips

Protein Tips with Affinity Resins



METTLER TOLEDO

Generic Guide for PureSpeed Set-up

1. Depending on the type of protein to be purified (His-tagged recombinant or full length IgG) choose the appropriate resin (IMAC, ProA or ProG) to optimize the purification process.
2. Determine the resin volume that will be required – higher resin volume will enable higher mass of protein to be captured and subsequently more available for analysis. Smaller resin volumes accomplish higher end product concentration.
3. Prepare the necessary buffers for the key steps in the process: Equilibration, Capture, Wash 1, Wash 2 and Elution. When using either ProA or ProG resins the protein being purified may require a neutralization step after the high acid elution step – be sure to have this prepared and ready to use.
4. Using the default program within the E4 XLS choose the appropriate resin type and volume. This program can be stored with an individual user name if needed. See page 8.
5. Using the Rainin ColorTrak Guide as a template, place the recommended 96-well deep well plate on top of the guide and add the appropriate volumes of buffers to each marked row.

Note: even if only purifying 2 samples, the number of channels on the pipette will determine which guide to use.
For single channel and 8-channel pipettes use the 8-channel side, for 12-channel pipettes, use the 12-channel side.
This preparation step should be checked before starting the purification process – make sure reagents are in each of the pre-marked rows for equilibration through to elution. Finally, add the sample to the row marked Capture.
6. Remove the buffer-filled deep-well plate and place the platform over the ColorTrak Guide – note the cut-outs in the guide should align with the platform's rubber feet. Place the deep-well plate on the platform and slide into position along the rails of the platform. The appropriate rows containing buffer should be aligned over the ColorTrak Guide.
7. With the sample and buffers now prepared, remove the appropriate clamshell with PureSpeed Tips containing the specific resin type and volume from the refrigerator ready for loading onto the E4 XLS pipette that has been prepared in step 4.
8. With the E4 XLS program for the appropriate PureSpeed function ready, press RUN on the E4 XLS softkey. The E4 XLS will instruct the user to install the appropriate tips.
9. Depending on the number of samples to be purified, prepare the appropriate pipette holding system. For single channel pipette use the Single Pipette Adapter. It should be placed over the well containing the Equilibration buffer. For multi-channel use, place two adapters (green adapters for 200 μ L tips and blue adapters for 1000 μ L tips) over the next-to-outer tips to secure the pipette/tip assembly in place in each of the rows.
10. Push each adapter fully onto the tip. Add the remaining tips to the pipette and press the CONT softkey on the E4 XLS. Follow the screen instructions and place the assembly into the Equilibrate row. The adapters will maintain the correct height for the tips in each stage of the process. In particular the elution step, with the lowest volume of buffer, requires the tips to touch the elution buffer and the adapters are manufactured to accommodate this.
11. With the assembly in place in the Equilibration buffer press the "CONT" softkey to continue the purification process and follow the instructions given on the E4 XLS screen.

1.0 Introduction

Rainin PureSpeed Protein Tips radically simplify purification of native or recombinant proteins. This innovative approach results in highly pure and concentrated purified protein, allowing many options for downstream functional assays. Up to 12 samples can be processed in parallel using Rainin's state-of-the-art E4 XLS multichannel electronic pipette in PureSpeed mode.

1.1 Storage

Store the tips at 4° C after receipt; all other PureSpeed components can be stored at room temperature. Glycerol that is added to the tips to protect the resin can form droplets on the tip walls during shipment, but this is normal and will not affect a tip's performance.

1.2 PureSpeed Tips and Accessories

PureSpeed Protein tips are offered with 3 different resins –

ProA for purifying IgG antibodies

ProG for purifying IgG antibodies

IMAC for purifying His-tagged recombinant proteins

These resins are offered in 2 different tip volumes, 200 µL and 1000 µL, each with 2 volumes of resins to accommodate a variety of sample volume needs. PureSpeed tips are supplied in "clamshells" to protect the resins, 12 tips to a clamshell. PureSpeed products are supplied in Starter Kits or as individual items.

Cat. No.	MT Order No	Description	Cat. No.	MT Order No	Description
PT-2-A5	17012561	5 µL ProA resin, 12 x 200 µL tips	PT-10-A20	17012568	20 µL ProA resin, 12 x 1000 µL tips
PT-2-A20	17012562	20 µL ProA resin, 12 x 200 µL tips	PT-10-A80	17012569	80 µL ProA resin, 12 x 1000 µL tips
PT-2-G5	17012563	5 µL ProG resin, 12 x 200 µL tips	PT-10-G20	17012570	20 µL ProG resin, 12 x 1000 µL tips
PT-2-G20	17012564	20 µL ProG resin, 12 x 200 µL tips	PT-10-G80	17012571	80 µL ProG resin, 12 x 1000 µL tips
PT-2-N5	17012566	5 µL IMAC resin, 12 x 200 µL tips	PT-10-N20	17012572	20 µL IMAC resin, 12 x 1000 µL tips
PT-2-N20	17012567	20 µL IMAC resin, 12 x 200 µL tips	PT-10-N80	17012573	80 µL IMAC resin, 12 x 1000 µL tips

PureSpeed Starter Kits (contain PT-ACC, E4 XLS and PureSpeed tips)

PT-S2-A20	17012577	Starter Kit, E4-200XLS, ProA	PT-S10-A20	17012579	Starter Kit, E4-1000XLS, ProA
PT-S2-G20	17012578	Starter Kit, E4-200XLS, ProG	PT-S10-G20	17012580	Starter Kit, E4-1000XLS, ProG
PT-S2-N20	17012576	Starter Kit, E4-200XLS, IMAC	PT-S10-N20	17012581	Starter Kit, E4-1000XLS, IMAC

PureSpeed Accessories

PT-ACC	17012588	PureSpeed Accessory Kit. Contents: Base, SD card, 96-deepwell plate, ColorTrak Guides, Adapters 200 µL, Adapters 1000 µL
PT-PT96	17012584	PureSpeed Base (in PT-ACC)
PT-E4-SD	17012587	E4 XLS Micro SD Card with PureSpeed protocol (in PT-ACC)
LR-P2-96P-5	17012623	96-deepwell plate, 5-pack (one supplied in PT-ACC)
PT-2-H	17012582	Pack of 10 adapters, for 200 µL multichannel (in PT-ACC)
PT-10-H	17012583	Pack of 10 adapters, for 1000 µL multichannel (in PT-ACC)
PT-2-S	17013242	Adapter for 200 µL single channel (in Starter Kit)
PT-10-S	17013226	Adapter for 1000 µL single channel (in Starter Kit)

1.3 PureSpeed Accessories

PureSpeed Accessories can optimize the purification process, allowing you to purify proteins in as little as 15 minutes.



In Accessory Kit:

1. Platform
2. 96-deepwell plate
3. Micro-SD card
4. 2 Mylar ColorTrak™ Guides for ProA /ProG and IMAC (2-sided)
5. 10 each multichannel adapters 200 μ L (green) & 1000 μ L (blue)

In Starter Kit:

6. One single channel adapter 200 μ L or 1000 μ L

Figure 1: PureSpeed Accessories

2.0 Set Up for Protein Purification with PureSpeed Protein Tips and E4 XLS Pipette

2.1 Buffer Preparation

ProA / ProG resins

Prepare a 5X buffer: 50 mM sodium dihydrogen phosphate, 700 mM sodium chloride, pH 7.4

Then dilute to 1X for the Equilibration and Wash 1 buffers.

Equilibration buffer: See above.

Wash 1 buffer: See above

1X Wash 2 buffer: Saline solution (140 mM NaCl)

1X Elution buffer: 200 mM sodium dihydrogen phosphate, 140 mM sodium chloride, pH 2.5

1X Neutralization buffer: 1 M Tris buffer, pH 9.0

Note: With PureSpeed ProA and ProG, we recommend using neutralization buffer after elution of the IgG. After the elution step, add neutralization buffer at 25% v/v of elution buffer volume. (eg: if protein eluted in 20 μ L elution volume, add 5 μ L neutralization buffer).

IMAC resin

Prepare a 5X buffer: 50 mM sodium dihydrogen phosphate, 1.5 M sodium chloride, 25 mM imidazole, pH 7.4

Then dilute to 1X for the Equilibration and Wash 1 buffers.

Equilibration buffer: See above

Wash 1 buffer: See above

5X Wash 2 buffer: 50 mM sodium dihydrogen phosphate, 1.5 M sodium chloride, 0.1M imidazole, pH 7.4

Note: Dilute the wash 2 buffer 5 fold to obtain 20 mM imidazole in the wash

1X Elution buffer: 200 mM sodium dihydrogen phosphate, 250 mM imidazole, pH 7.4

Note: For high protein yield, use up to 0.5 M imidazole and 0.5 M NaCl.

However, this may lead to poor protein purity.

2.2 Purification Set-up

To remove sample particles, spin sample and remove supernatant for processing in PureSpeed tips.

As a guide, set up samples and buffers in a deepwell plate as follows:

Row 1 – Equilibration buffer

Row 2 – Samples including negative and positive controls, if any

Row 3 – Wash 1

Row 4 – Wash 2

Row 5 – Elution buffer

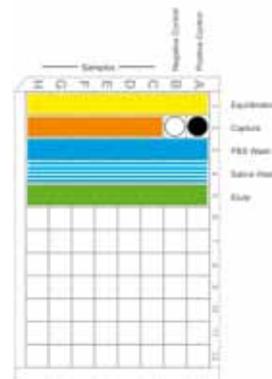


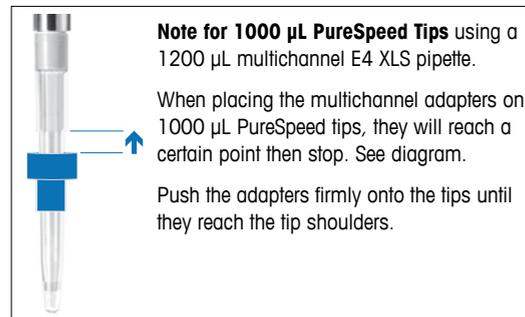
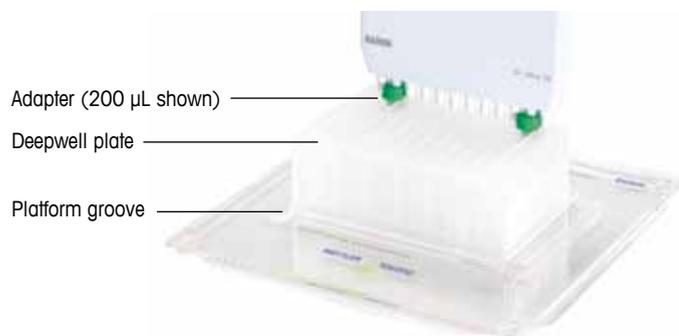
Figure 2: Deepwell Plate Set up for antibody purification using ProA resin

Chart showing various standardized protocol buffer volumes

Resin Type	Pro A				Pro G				IMAC			
	200 μ L		1000 μ L		200 μ L		1000 μ L		200 μ L		1000 μ L	
Resin μ L	5	20	20	80	5	20	20	80	5	20	20	80
Equilibration μ L	100	100	500	500	100	100	500	500	100	100	500	500
Capture sample μ L*	10-200	10-200	100-1000	100-1000	10-200	10-200	100-1000	100-1000	10-200	10-200	100-1000	100-1000
Wash I μ L	100	100	500	500	100	100	500	500	100	100	500	500
Wash II μ L	100	100	500	500	100	100	500	500	100	100	500	500
Elution μ L	15	60	60	240	15	60	60	240	15	60	60	240

*For larger samples use multiple aliquots in the Capture step (e.g. Capture 2x, Capture 3x, etc.)

Take the deepwell plate and slide it into the grooves on the base as shown below. The figure shows a 12-channel pipette in use: note the adapter positions on the 2nd and 11th tips. Depending upon the E4 XLS model you are using, your set-up may be different.



Note for 1000 μ L PureSpeed Tips using a 1200 μ L multichannel E4 XLS pipette.

When placing the multichannel adapters on 1000 μ L PureSpeed tips, they will reach a certain point then stop. See diagram.

Push the adapters firmly onto the tips until they reach the tip shoulders.

Figure 3: PureSpeed Accessory Kit Assembled

2.3 Installing the micro SD card into the E4 XLS

The PureSpeed mode will only operate with E4 XLS LTS 200 μL and 1000 μL single channel and 200 μL and 1200 μL multichannel models. Open the battery compartment on the E4 XLS, remove the battery and locate the micro SD slot, referring to the diagram on the inside of the battery compartment. See Figure 4. Take the micro SD card from the kit and slide it into the slot in the orientation shown on the diagram inside the battery compartment.



Figure 4A: Micro SD slot



Figure 4B: Micro SD card in slot

When the micro SD card is properly aligned in the slot, push the card until it locks in place (you may need to use a flat bladed screwdriver to gently but firmly push it in). Replace the battery. The E4 XLS will detect the micro SD card and activate the PureSpeed mode. Replace the battery cover.

3.0 Using E4 XLS in PureSpeed Mode

The PureSpeed mode is activated in the E4 XLS pipette when a PureSpeed enabled SD card is inserted into the card slot and E4 XLS is restarted. The PureSpeed icon will appear in the carousel.

This manual assumes that you have a basic understanding of protein purification methods and that you are familiar with the E4 XLS electronic pipette (see also E4 XLS manual Part No. 9920-396). When describing E4 XLS, the movements and actions of the joystick control are represented herein by the following icons:

● press joystick ▲ move joystick up ▼ move joystick down ◀ move joystick left ▶ move joystick right

The terms Speed, Aliquot, Step and Cycle have special meanings in this manual:

Speed: LOW, MEDIUM and HIGH result in the following flow rates:

Tip Volume	LOW	MEDIUM	HIGH
200 μL	50 $\mu\text{L}/\text{min}$	250 $\mu\text{L}/\text{min}$	500 $\mu\text{L}/\text{min}$
1000 μL	250 $\mu\text{L}/\text{min}$	500 $\mu\text{L}/\text{min}$	1000 $\mu\text{L}/\text{min}$

Aliquot: Number of discrete liquid samples per purification step

(Wash) Step: Number of discrete wash steps in the purification process.

Cycle: One complete aspiration and dispense.

3.1 Protocol Selection

With the PureSpeed icon visible, press ●, ▲ or ▼ to launch PureSpeed mode. The next screen is the Protocol Select screen, where protocols are defined by the type of PureSpeed tip to be used. The top field allows selection by resin type ("IMAC" in the example) and resin bed size ("20 μL " in the example). Under the Tip Type field is a list of up to 20 user-defined saved protocols. If no protocols have been saved, only the "DEFAULT" indicator is displayed.

To change tip type, press ● or ▶ to activate the tip type field. Once activated, the value can be changed by pressing ▲, ▼, ◀ or ▶. Each time the tip type is changed, the "Saved Protocol" box is updated with the list of previously saved protocols.



When the tip type field is active, pressing  or the "SELECT" soft key selects the tip type shown and activates the "Saved Protocols" scrollable box.

Select the desired protocol name by pressing  or . Press the "SELECT" soft key or press  to load the selected protocol. If "DEFAULT" is selected, the default values for the selected tip type will be loaded. Pressing "CANCEL" will restore the original selection and return focus back to tip type selection.

3.2 Protocol Setup

After selecting the correct protocol the protocol setup screen appears. The PureSpeed system is designed to function in four distinct steps: Equilibration, Capture, Wash and Elute. The Protocol Setup screen enables you to view and modify the parameters in each step. In the Protocol Setup screen, press  or  to select the step to view. Press  or  to view or modify the step parameters.

Hint: The selected tip type appears in the upper right portion of the screen under the battery icon. The selected saved protocol appears in the lower left above the "BACK" soft key. The time required to run the complete protocol appears in the lower right in HH:MM:SS format. This value is updated each time settings are changed.

Hint: Each step is assigned a color that appears here and in the step prompt icons. These colors can be used in conjunction with the ColorTrak Guides to facilitate steps and reduce errors.

Once a step is selected a "pop-up" box presents the parameters and settings. Press  or  to select a particular parameter. Each tip type establishes parameter boundaries, limiting the range of settings. To activate a field, press  or . An active field is highlighted white.

NOTE: The defined protocol may limit some parameters to a fixed setting. In these cases, the values cannot be selected.

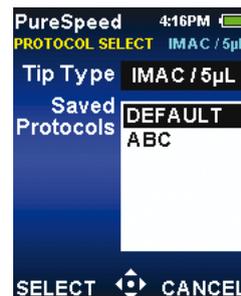
Hint: Pressing the  "NEXT" soft key in the setup screen advances to the next step. This feature also provides a quick way to review all steps.

You can change an active field's value by pressing  or . To change larger increments use  or . Once a parameter is set to the desired value, press  or the "DONE" soft key to save the change. Pressing the "CANCEL" soft key will leave the field unchanged.

Hint: The total time required to complete the protocol is displayed in the lower right above the right soft key in "HH:MM:SS" format (the example indicates 9 minutes and 32 seconds). This value is updated after each parameter change.

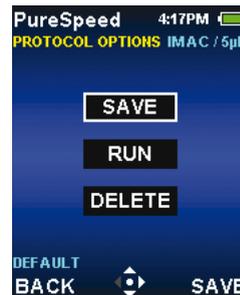
Hint: It is possible to bypass a step by entering a zero in the Aliquots field.

Hint: The "NEXT" soft key allows you to quickly index through all the step settings.



3.3 Protocol Save

Once all steps are configured properly, the "DONE" key returns you to the main protocol setup screen. Then the "NEXT" soft key advances to the "Protocol Options" screen. Here the option to "SAVE" or "RUN" the protocol is given. If changes have been made and will be needed in the future, it is advisable to save the protocol at this time. Deleting a previously-saved protocol is also an option. See section 3.5.



Pressing ● or ► activates the "Protocol Save" selection screen. By default, the active protocol is highlighted. Press "SELECT" to save the settings to the current protocol definition.



The second save screen presents the user with the selected save name and a "SAVE" button.

Hint: PureSpeed mode provides space for up to 20 saved protocols per tip type. Once this allocation is full, use the delete function to make space for new protocols.

To save the protocol to a different name, press ▲ to select the "Save Name" field and press ● or ► to activate the name field. Once activated, you can edit the name. Use ▲ or ▼ to select the letter or number. Then ► for next symbol. You can clear the current name with "CLEAR". After the name is completed, press "DONE" or ● to save. Pressing the "BACK" soft key will return to the Protocol Options screen.



To perform the save operation, select the "SAVE" field and press ● or ► to activate it. Once the save is completed, the E4 XLS will return to the protocol options screen.

Hint: The active protocol name displayed in the lower left of the screen is always the last protocol saved. If the default settings are active, the name will appear as the tip identifier (resin type/bed size).



3.4 Protocol Execution

To run the protocol, select the run screen button on the Protocol Options screen and activate it by pressing ● or ► or press the "RUN" key. Activating the run option will bring up the run screen, which is similar to the other E4 XLS pipette screens. The step name is displayed in yellow in the sub mode area of the screen. The familiar green boxes on the right portion of the screen display the step setup data. Cycle and aliquot numbers will update as the protocol progresses.

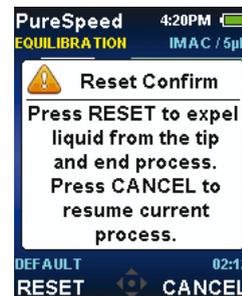
The E4 XLS will instruct the user to install the proper tips. Press the "CONT" soft key when the appropriate tips have been mounted.

See page 4 for preparing the 96-deepwell plate and loading tips.

When the unit is ready to begin operation, another pop-up message will instruct the user to place the pipette into the Equilibration solution. Again, press the "CONT" soft key to start this cycle – verify the process is operating.

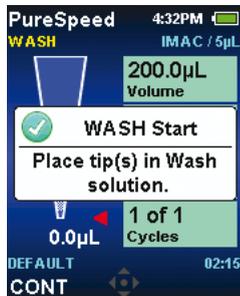


During protocol execution, the process can be paused at any time by pressing the "PAUSE" soft key – any motion or timeouts are paused. The soft keys present an option to "RESET" or to "RESUME" operation. If "RESET" is pressed, a confirmation message pops up asking to confirm the reset or to "CANCEL" the request. A reset terminates the active protocol by dispensing the liquid in the tip. A second prompt alerts to remove the tip (which can then be reused). The E4 XLS then completes the reset and performs a blowout operation.



Hint: In RUN mode, the time value that appears in the lower right is the active number of minutes and seconds until the next intervention prompt.

When the Equilibration step is complete, the unit prompts the user to move the tip to the Capture solution, and then to the Wash and Elution solutions. The prompts for each step are color coded to match the rows in the ColorTrak Guides. The intervention prompts will generate a warning beep and screen flash along with the pop-up message. The message will re-activate lighting the screen and generating additional beeps at 60 second intervals until the prompt is answered.



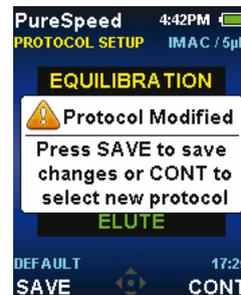
If multiple aliquots or steps are required, the unit will prompt to indicate the start of each new aliquot or step:



After the final step is complete (normally the Elute step), the pipette will prepare for reset by prompting the user to remove the tips. Once the "CONT" soft key is pressed, the pipette will reset and return to the options screen.

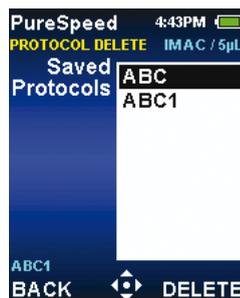


To exit the run mode press the "BACK" soft key. This will return to the protocol steps screen. Pressing "BACK" at this level will return to the Protocol Select screen. If protocol edits have been made that were not saved, the unit will prompt the user to save them.



3.5 Deleting Saved Protocols

Saved protocols that are no longer needed can be deleted by activating the delete button on the Protocol Options screen. Pressing  or the "DELETE" soft key will bring up the Protocol Delete page. NOTE: If no protocols are currently saved, the "Delete" option will not appear. The protocol delete page displays saved protocols that can be deleted. Press  or  to select the protocol to delete and then press "DELETE".



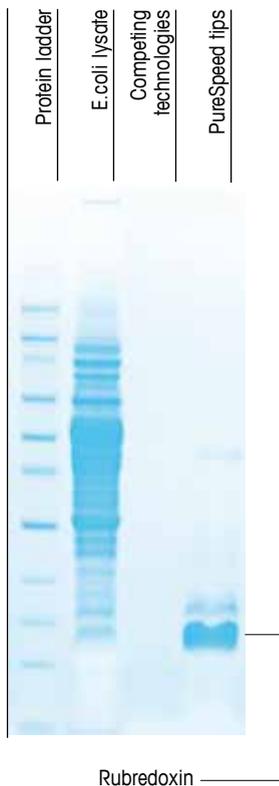
Once "DELETE" is pressed, a pop-up message will confirm the delete operation. Press the "DELETE" softkey to verify that the protocol is to be deleted. Once the delete is complete the E4 will return the Protocol Options page. Repeat this operation to delete multiple protocols.

PureSpeed Protein Tip Data

PureSpeed Protein Tips provide the highest concentration of pure functional protein when compared with conventional spin column methods:

A large E. coli lysate sample was spiked with an unknown amount of His-tagged rubredoxin (MW 7500). 100 μ L of lysate was purified by either spin column or PureSpeed IMAC tips packed with 5 μ L resin. 10 μ L of the resulting eluted samples were loaded onto SDS-PAGE and the gel was stained by coomassie blue.

As shown in the gel, PureSpeed tips outperformed spin column both in terms of purity and concentration.



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