User Manual

Pierce Power Stainer
Pierce Power Blotter
Pierce Power System

Catalog Number 22830, 22833, 22834, 22835, 22836, 22838

Publication Number MAN0014886

Publication Part Number 2162585.1

Revision A.0

Thermo SCIENTIFIC

Table of Contents

1

Chapter 1: Overview

The Thermo Scientific™ Pierce™ Power Stainer (#22833) consists of a Thermo Scientific™ Pierce™ Power Station (#22838) with staining software and a Thermo Scientific™ Pierce™ Power Stain Cassette (#22836) for rapid Coomassie staining and destaining of proteins in polyacrylamide gels. Traditional Coomassie staining techniques require one hour to overnight for complete staining and destaining, but, when used in conjunction with Thermo Scientific™ Pierce™ Midi and Mini Gel Power Staining Kits, the Pierce Power Stainer provides staining plus destaining efficiency in as few as 6 minutes. This significant reduction in protein staining/destaining time is accomplished by fixing the protein to the gel and electrophoretically transporting the negatively charged Coomassie R250 dye rapidly through the gel matrix. The dye passes through the polyacrylamide and ionically binds to the protein, resulting in crisp blue bands with minimal background. With the system pulling remaining dye through the gel, no destaining is required. The system has been verified to work with commonly used pre-cast and homemade SDS-PAGE gels.

The Thermo Scientific™ Pierce™ Power Blotter (#22834) consists of the Pierce Power Station with blotting software and the Thermo Scientific™ Pierce™ Power Blot Cassette (#22835) for rapid semi-dry transfer of proteins from polyacrylamide gels to nitrocellulose or PVDF membranes. Traditional Western blotting techniques require a transfer of one hour to overnight to achieve optimal transfer efficiency. When used in conjunction with Thermo Scientific™ Pierce™ 1-Step Transfer Buffer, the Pierce Power Blotter provides transfer efficiency in 5-10 minutes, which is equivalent to, or better than, traditional blotting techniques without the need for gel pre-equilibration. This significant reduction in protein transfer time is accomplished by optimizing the ionic strength of the transfer buffer and increasing the current [amps (A)/cm^2] flowing through the transfer stack. The system has been verified to work with commonly used pre-cast and homemade SDS-PAGE gels. The Pierce Power Blotter can also be used for standard semi-dry transfer protocols with Towbin buffer.

The Thermo Scientific™ Pierce™ Power System (#22830) consists of a Pierce Power Station with staining and blotting software, and the combination of the Pierce Power Stain Cassette and the Pierce Power Blot Cassette.

Contacting Thermo Fisher Scientific

For technical or service assistance on the Pierce Power Stainer, Blotter or System:

- U.S. Customers: 1-800-955-6288

  Fax: 1-800-331-2286

  Technical Support email for U.S. customers: customerservice@lifetech.com

- International Customers: Go to www.lifetech.com/ordersupport and select your specific location from the drop-down list at the bottom of the page. Selecting your country will provide contact and technical service details for local distributors.

For more information on the Thermo Scientific Protein Biology product line, please visit thermofisher.com

Instrument Overview

Unpacking and Setup Instructions

Unpack your new Pierce Power Station and/or cassette(s).

Verify that all of the accessories listed below are included with your package. Please note that the Pierce Power Station and cassette(s) can be purchased separately or combined.

Your purchase includes the following accessories (each quantity 1):

Included Accessories (Power Stainer, #22833)

Pierce Power Station
Pierce Power Stain Cassette
Quick Start Guide
Roller
Power Cord with C13 Connector

Included Accessories (Power Blotter, #22834)

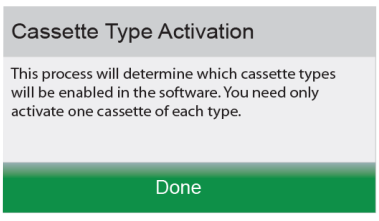
Pierce Power Station
Pierce Power Blot Cassette
Quick Start Guide
Roller
Power Cord with C13 Connector

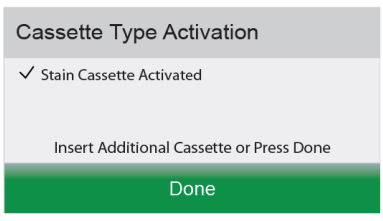
Included Accessories (Power System, #22830)

Pierce Power Station
Pierce Power Stain Cassette
Pierce Power Blot Cassette
Quick Start Guide
Roller
Power Cord with C13 Connector

User Interface

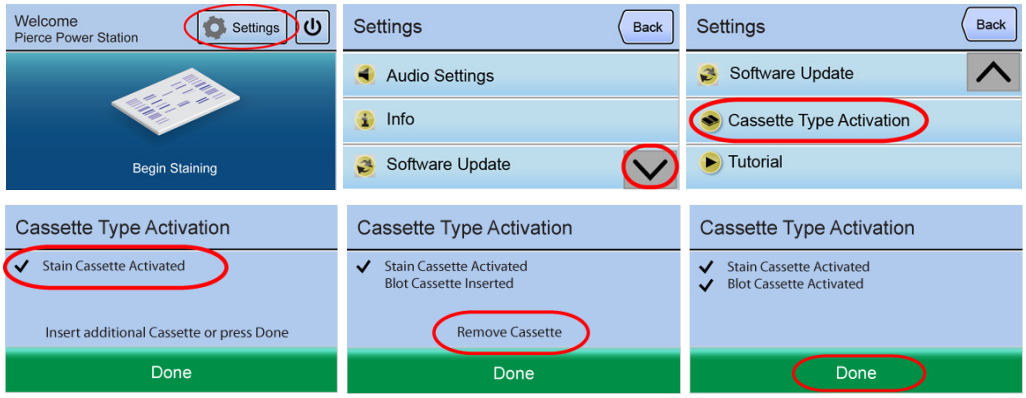
The user interface consists of a color LCD menu touchscreen on the top of the Pierce Power Station. When turning on the Pierce Power Station for the first time, the **Cassette Type Activation** screen will appear. Follow the directions on the screen and insert the Power Stain Cassette or the Power Blot Cassette. The appropriate software will be activated and the option of inserting an additional cassette or pressing **Done** is given. Insert additional cassette or press **Done**.



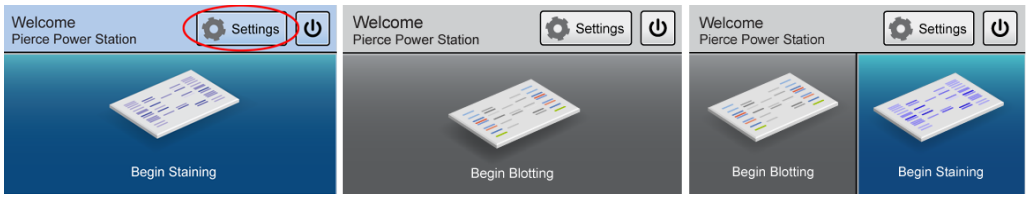


IMPORTANT If one cassette type (Power Stain Cassette or Power Blot Cassette) was previously activated, then the new cassette type must also be activated with the Pierce Power Station.

For example, when using the Pierce Power Blot Cassette for the first time with the Pierce Power Station activated only for staining, the blotting software must be activated. Press the **Settings** button on the Welcome screen. Press the arrow down to view more menu options, and select the **Cassette Type Activation** button. Currently installed cassette types will show up on the screen with a check mark to the left. To activate the blotting software, insert the Pierce Power Blot Cassette into the Pierce Power Station and remove when instructed. Press **Done** or activate an additional cassette type.



From the **Welcome Pierce Power Station** screen, the following selections are possible:

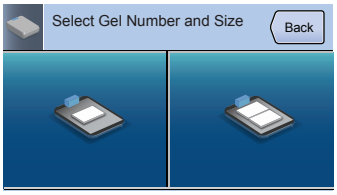


1. **Settings button** – change audio settings, view software and hardware versions, install software updates to the user interface, activate cassettes, and access Tutorial for blotting and staining.
2. **Shutdown button** – safely shuts down the device.
3. **Begin Blotting** – allows you to quickly access **Pre-Programmed Methods**, **Recent Methods** and **Custom Methods**, or return to **Main Menu**.
    a. **Pre-Programmed Methods** – allows you to quickly access the recommended amperage, voltage and time for the size and number of gels that you intend to simultaneously transfer. The blotter is intended for use only with the following consumables:
        • Pierce 1-Step Transfer Buffer (Product #84731)
        • Bio-Rad™ Trans-Blot™ Turbo™ Transfer Stacks
        • Towbin Transfer Buffer for Standard Semi-Dry Transfer (only)
    b. **Recent Methods** – gives you access to recently run methods listed from most to least recent.
    c. **Custom Methods** – creates, runs and saves a custom transfer method.
4. **Begin Staining** – allows you to quickly access **Select Gel Number and Size** or return to Main Menu.

The Pierce Power Stainer is intended for use only with the Pierce Midi Gel Power Staining Kit (#22839) and Pierce Mini Gel Power Staining Kit (#22840) that contain the following consumables:

- Power Stain Solution
- Destain Solution
- Mini or Midi Gel Pads (Pads consist of eight sheets of white material placed together in like orientation. Each pad is separated by a blue interleaf.)

a. **Select Gel Number and Size** – allows you to choose the option of staining one mini-size gel, two mini-sized gels or one midi-sized gel. Touching the desired application will lead you to **Choose Method**.

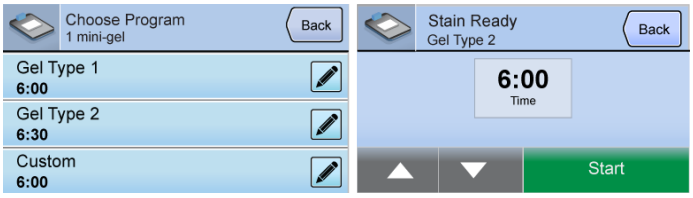


b. **Choose Method** – allows you to quickly access the recommended program for commonly used SDS-PAGE gels. Refer to Table 1.1.

Table 1.1. Recommended staining methods.

Gel Description	Gel Number and Size	
	1 Mini Gel	**2 Mini Gels or 1 Midi Gel**
NuPAGE™ Bolt™ Bis-Tris Mini Gel Novex™ NuPAGE Bis-Tris Mini Gel Mini-PROTEAN™ TGX™ Gel	Type 1, 6:00	Type 1, 6:30
Novex Tris-Glycine Mini Gel Homebrew Tris-Glycine Mini Gel	Type 2, 6:30	Type 2, 11:00
Novex NuPAGE Bis-Tris Midi Gel Novex Tris-Glycine Midi Gel Criterion™ Tris-HCl Gel	---------- ---------- ----------	Type 2, 11:00

c. The pen button will allow you to **Edit Name** and **Modify Method** (method name and parameters will be displayed in *italics*). Press **Restore Default** on the Change Method screen to revert any modified methods back to preinstalled settings. Select appropriate method to proceed to Stain Ready screen. **Stain Ready** allows you to change the time (minimum 30 seconds, maximum 12 minutes). Press **Start** to initialize staining. Press **Pause/Stop** to interrupt the staining program.



2

Chapter 2:
Installation Guidelines

IMPORTANT Review and implement guidelines for proper set-up before installation. Locate the Pierce Power Station away from water, solvents, corrosive materials, strong magnetic fields and vibration sources.

Space Requirements

The Pierce Power Station requires a stable laboratory bench or table. Provide a minimum clearance of 3 inches (7.62cm) at the rear of the Power Station to allow for adequate ventilation for cooling and access to the power cord and switch. In addition, allow sufficient space at the front of the stainer for docking and removing the cassette with/from the control unit.

Table 2.1. Thermo Scientific Pierce Power Station dimensions.

Dimensions	Inches	Centimeters
Width	6.4	16.4
Depth	10.0	25.3
Height	1.4	3.5

Table 2.2. Thermo Scientific Pierce Power Stain Cassette dimensions.

Dimensions	Inches	Centimeters
Width	6.4	16.2
Depth	10.0	25.3
Height	1.4	3.5

Table 2.3. Thermo Scientific Pierce Power Blot Cassette dimensions.

Dimensions	Inches	Centimeters
Width	10.1	25.7
Depth	11.0	27.8
Height	1.4	3.5

Electrical Requirements

Note A grounded circuit capable of delivering the appropriate current and voltage is required for installation. Electrical requirements can be located on the rear panel of the stainer. The Power Station electrical system will adjust to the proper voltage for the respective country. Connect the power cord to the rear left-side panel of the device and plug into a grounded power outlet.

WARNING After running at high current, the anode and cathode plates can become hot. Use caution when separating the gels and stacks from the plates. When continuously processing multiple samples for a maximum of 2 hours, allow the cassette to cool for 30 minutes or use multiple cassettes to avoid excessive cassette heating.

WARNING Pierce Power Station used outside of the workflows described in this manual may put the operator at risk of dangerous exposure to electrical shock. Do not use this instrument for any purposes or in any configurations not described in this manual.

WARNING The Pierce Power Station can contain dangerous electricity and is not designed to be opened by the user. Disconnect all power to the control unit before maintenance by a qualified technician.

WARNING Do not overfill the cassettes with liquid. Excess liquid can overflow into the control unit and possibly cause electric shock. Follow the appropriate instructions for reagent amounts and empty any remaining liquid in the cassette upon run completion.

Table 2.4. Thermo Scientific Pierce Power Station electrical ratings.

Pierce Power Station Electrical Parameter	Rating
Supply Voltage (VAC)	6.44
Frequency (Hz)	10.0
Maximum Power Rating (W)	1.38
Fuse (Power Center)	T4AL, 250V, 4A

Table 2.5. Thermo Scientific Pierce Power Station electrical requirements for major countries.

Region	Voltage (VAC)	Frequency (Hz)	Amps (A)
US and Canada	120	60	1.0
Europe	220	50	0.5
UK	240	50	0.5
Japan	100	50	1.2
China	220	50	0.5
India	230	50	0.5
South Korea	220	60	0.5

Environmental Requirements

For optimal performance, maintain a constant laboratory temperature range of 15-25°C (59-77°F) with relative humidity between 20-70%. Do not place the Pierce Power Station in direct sunlight.

Power Station Images with Feature Locations

Figure 2.1. Thermo Scientific Pierce Power Station front and side views showing main components of control unit.

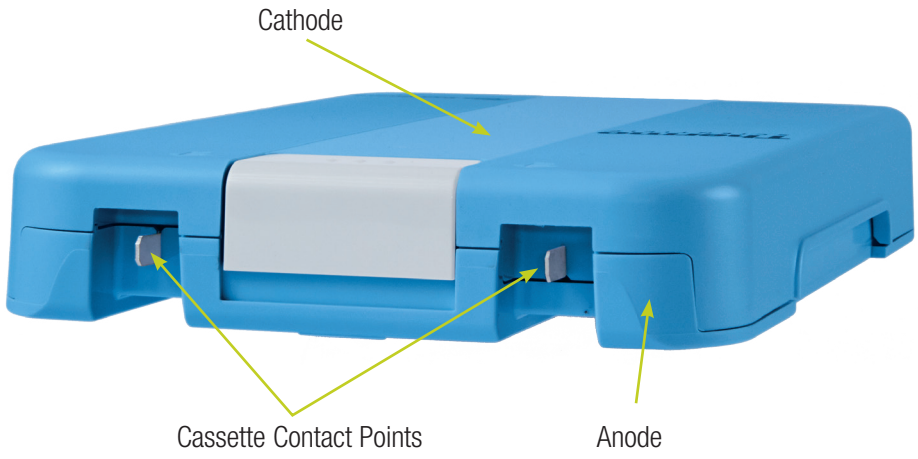


Figure 2.2. Thermo Scientific Pierce Power Blot Cassette view showing the upper cathode, the lower anode and the electrical contacts that insert into the Thermo Scientific Pierce Power Station contacts. The Thermo Scientific Pierce Power Stain Cassette has similar feature locations.

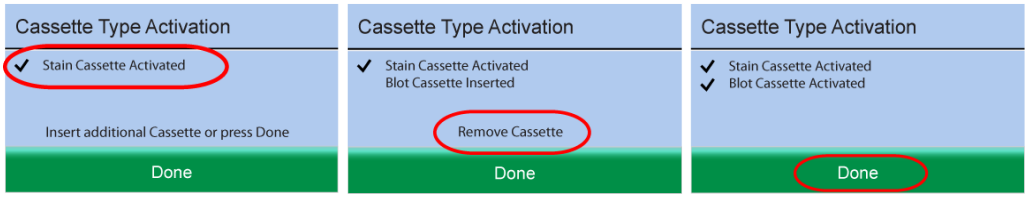
Figure 2.3. Thermo Scientific Pierce Power Station with Thermo Scientific Power Stain Cassette inserted.

Figure 2.2. Rear view of Thermo Scientific Pierce Power Station.

Start Up

Turn the power switch located at the bottom rear of the stainer panel to the **On** position. The power switch provides power to all of the stainer/blotter components. The on-board computer boots up, the touchscreen displays the Thermo Scientific logo followed by "Welcome to Thermo Scientific Pierce Power Station," and then automatically moves to Welcome Pierce Power Station and the **Main Menu**. The Pierce Power Station is now ready for operation.

IMPORTANT When turning on the Pierce Power Station for the first time, the **Cassette Type Activation** screen will appear. Follow the directions on the screen and insert either the Pierce Power Stain Cassette or the Pierce Power Blot Cassette. The appropriate software will be activated and the option of activating an additional cassette or pressing **Done** is given. Insert an additional cassette or press **Done**. When using the Pierce Power Stain Cassette for the first time with the Pierce Power Station currently activated for blotting only, you must activate the staining software. Press the **Settings** button on the Welcome screen. Press the arrow down to view more menu options, and select the **Cassette Type Activation** button. Currently installed cassette types will show up on the screen with a check mark to the left. To activate the staining software, insert the Pierce Power Stain Cassette into the Pierce Power Station and remove when instructed. Press **Done** or activate an additional cassette type.



Shut Down

On the **Main Menu** screen, touch the **Shut Down** button. When prompted "Are you sure you want to shut down?" select **Yes**. When notified that "It is now safe to turn off the device," turn the power switch to the **Off** position.

3

Chapter 3:

Pre-Programmed Staining Methods

Electrophoretic Staining

Electrophoretic staining fixes the protein to the gel and electrophoretically transports the negatively charged Coomassie R250 dye rapidly through the polyacrylamide gel matrix. The dye ionically binds to the protein, resulting in crisp blue bands with minimal background. The system has been verified to work with commonly used pre-cast and homemade SDS-PAGE gels.

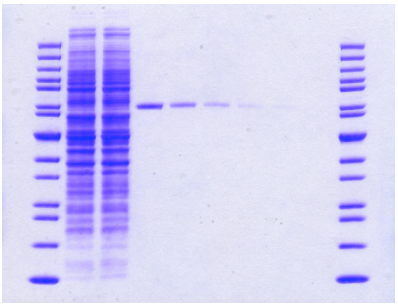


Figure 3.1. Gel stained using electrophoretic staining protocol (dynamic range).

The traditional Coomassie protocol uses gel pre-equilibration or a fixing step and longer staining and destaining times to achieve desirable results.

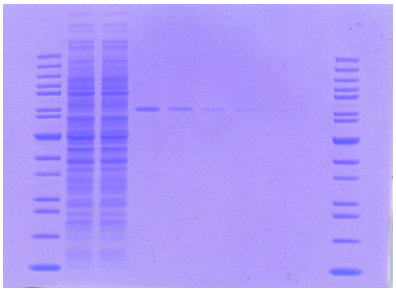


Figure 3.2. Gel stained using traditional Coomassie protocol (5-hour water wash).

Pierce Power Stain Solution, Destain Solution and Gel Pads

Electrophoretic staining/destaining is designed to stain/destain one to two mini-sized gels or one midi-sized gel in as few as 6 minutes. Gels stained/destained simultaneously must have the same formulation. Mini gels are defined as ~46-60cm^2. Midi gels are defined as ~110cm^2. Note that these dimensions are the surface area of the gel without the gel fingers (not of the cassette).

Every gel type will require process optimizations while performing the staining/ destaining steps. To ensure optimal results, follow the appropriate instructions.

Note Staining/destaining of 1-2 mini-sized gels will require time optimization (approximately 2/3 time is required compared to a full mini-sized gel of the same formulation).

1. Each gel requires two multilayer gel pads. Each multilayer gel pad contains eight sheets of white fabric separated by a blue interleaf on top and bottom.

   Note We do not recommend using other material (such as Western blotting filter paper) in conjunction with the Pierce Power Stainer.

2. Prepare destaining (bottom) pad: place one multi-layered gel pad into a tray and add Destain Solution evenly to pad. Use 15mL of Destain Solution for mini gel pads and 30mL for midi gel pads.

   Note Pads can be prepared 5 minutes before use. If the pads are prepared earlier, cover the dishes containing the pads to eliminate potential evaporation.

3. Prepare staining (top) pad: place one multi-layered gel pad into a tray and add Power Stain Solution evenly to pad. Use 15mL of Power Stain Solution for mini gel pads and 30mL for midi gel pads.

   Note Pads can be prepared 5 minutes before use. If the pads are prepared earlier, cover the dishes containing the pads to eliminate potential evaporation.

4. After electrophoresis, remove gel(s) from cassette(s) and wash mini-sized gel 1 x 5 minutes and midi-sized gel 2 x 5 minutes in deionized water. When staining two mini gels simultaneously, wash each gel 2 x 5 minutes in deionized water.

5. Place the destaining pad on the center of the cassette bottom (anode). Use a roller to remove any trapped air bubbles.

   Note Removal of trapped air bubbles is essential for high-quality staining. Two firm passes with the roller (or other suitable utensil) is typically sufficient.

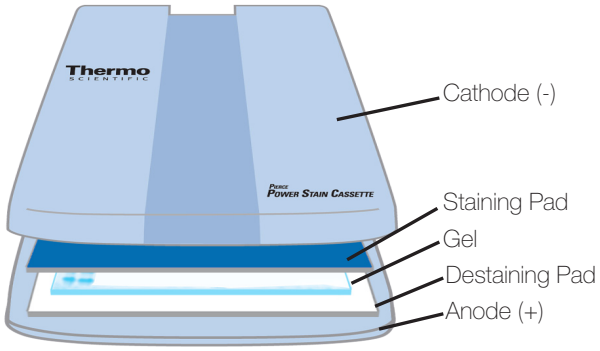
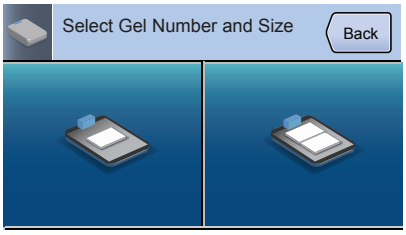


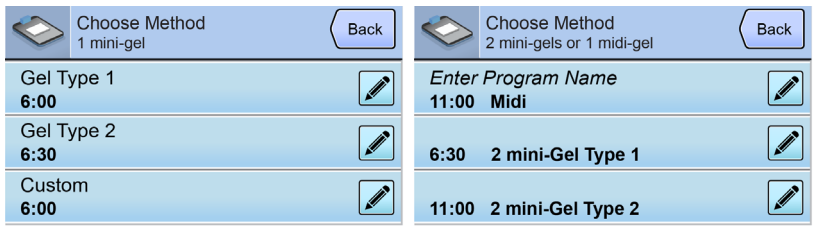
Figure 3.3. How to assemble the power stain sandwich in the stain cassette.

Note If assembling more than one stack on the anode surface, evenly space and center the sandwiches so the top cathode surface applies even pressure to the surface areas of the stack(s). Ensure there is a 1cm space around all stack edges.

6. Place the pre-washed gel on top of the destaining pad and use roller to remove any trapped air bubbles.
7. Place the staining pad on top of the gel and use roller to remove any trapped air bubbles.
8. Place cassette top (cathode) on top of cassette bottom (anode) and gently press down to lock into place.
9. Slide cassette into the Pierce Power Station and select **Begin Staining**.
10. Select appropriate icon in the **Select Gel Number and Size**.



11. In **Choose Method**, select the appropriate program to stain the gel(s):
    a. Programs for staining mini-sized gels:
        a. Gel Type 1 (6:00)
        b. Gel Type 2 (6:30)
        c. Custom (6:00)
    b. Programs for staining midi-sized or two mini-sized gels:
        a. Midi (11:00)
        b. 2 Mini-Gel Type 1 (6:30)
        c. 2 Mini-Gel Type 2 (11:00)

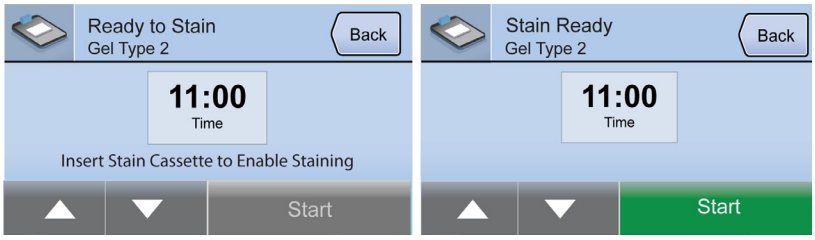


Note Refer to Table 1.1, pg. 6 for recommended staining methods.

12. Use **Pencil Button** to **Modify Method** and/or **Edit Name**, or to **Restore Default** staining parameters.

    Note Once an optimal method is found, it can be set as a custom method for future use.

13. Press the **Start** button to begin staining.



14. Upon staining completion, remove the cassette from the Pierce Power Station and open the cassette. Carefully remove the top staining pad from the stack and determine if the gel is sufficiently destained or requires additional destaining time.

    Note If the gel requires additional destaining time, reassemble the stack, use the roller to eliminate bubbles, place the cathode back on and insert the cassette into the Pierce Power Station. Set the time for 1:00 minute or 1:30 minutes and press **Start**.

    Note Thoroughly rinse cassette(s) under running water after each use. Hang to dry on a drying rack.

    Note Store stained gel in plastic protective sheet or in water for up to 4 hours.

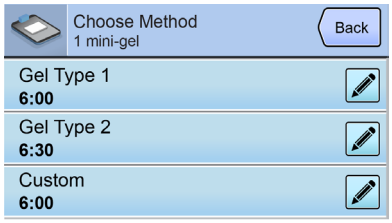
WARNING After running at high current, the anode and cathode plates can become hot. Use caution when separating the gels and stacks from the plates. When continuously processing multiple gels for a maximum of 2 hours, allow the cassette to cool for 30 minutes or use multiple cassettes to avoid excessive cassette heating.

4

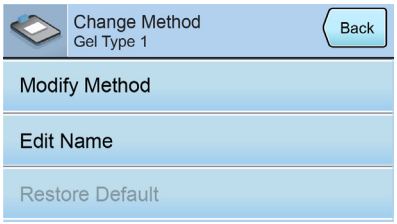
Chapter 4:

Modifying Staining Program Methods

1. Default methods can be modified in **Choose Method** within the **Gel Type** programs.



2. Pressing the **Pencil** button gives options to **Modify Method**, **Edit Name** or **Restore Default**.



3. Selecting **Modify Method** will allow you to lower or increase the time in 30-second increments (minimum allowable time is 30 seconds and maximum time is 12 minutes). After the program is modified, press the **Done** button.

   Note **Modifying Method** will change the font to italics. Pressing **Restore Default** will restore all default parameters.

4. Selecting **Edit Name** will allow you to change the name of the program (enter up to 15 characters). After the program is modified, press the **Done** button.

   Note Changing the program name will change the font to italics. Pressing **Restore Default** will restore all default parameters.

   Note Once an optimal method is found, it can be set as a custom method for future use.

Troubleshooting: Electrophoretic Staining

Problem	Possible Cause	Solution
Inconsistent destain	Inefficient washing of gel	Wash mini gel at least 1 x 5 minutes and midi gel 2 x 5 minutes in water. When staining two mini gels simultaneously, wash each mini gel 2 x 5 minutes in water
	Insufficient staining / destaining time	Add an additional 30 seconds to 1 minute of staining/destaining time or destain the gel in water for additional time
	Air bubbles trapped between gel and the pads	When assembling staining stack, use a roller or pipette to remove any air bubbles between the gel and the pads
Gel over-destained	Stain time too long	Decrease stain time by 30 seconds or 1 minute
Nonspecific spots or splotches of stain in polyacrylamide background not associated with protein	Inefficient washing of gel	Wash mini gel at least 1 x 5 minutes and midi gel 2 x 5 minutes in water. When staining 2 mini gels simultaneously, wash each mini gel 2 x 5 minutes in water
	Insufficient staining/ destaining time	Add additional 30 seconds to 1 minute of staining/destaining time or destain the gel in water for additional time
	Air bubbles trapped between gel and pads	When assembling staining stack, use a roller or pipette to remove any air bubbles between the gel and pads

5

Chapter 5:

Pre-Programmed Blotting Methods

Pierce 1-Step Transfer Buffer

Simultaneously transfer one to four mini-sized gels or one to two midi-sized gels in 5-10 minutes. Gels simultaneously transferred must have the same formulation. Mini gels are defined as ~46-60cm^2. Midi gels are defined as ~110cm^2. Note that these dimensions are the surface area of the gel with the gel fingers removed (not of the cassette).

1. Cut four sheets of ~0.83mm thick Western blotting filter paper and one sheet of nitrocellulose or PVDF membrane to the same size as the gel(s).

   Note The transfer stack should contain two sheets of ~0.83mm thick Western blotting filter paper on the bottom (anode), followed by membrane, gel and two sheets of ~0.83mm thick Western blotting filter paper on top. If 0.83mm thick filter paper is not available, do not exceed 1.8mm total filter paper thickness for the bottom of the stack or 1.8mm total filter paper thickness for the top of the stack. Use the appropriate Thermo Scientific™ filter papers:

   Product #84873, 0.83mm thick, 7.0cm x 8.4cm (mini-size)
   Product #84874, 0.83mm thick, 8.0cm x 13.5cm (midi-size)
   Product #88600, 0.83mm thick, 8.0cm x 10.5cm

2. Equilibrate filter paper and membrane in Pierce 1-Step Transfer Buffer for a minimum of 5 minutes. Use sufficient buffer to completely cover the filter paper and membrane.

   Note PVDF membrane must be wetted with methanol or ethanol before equilibration in Pierce 1-Step Transfer Buffer.

   Note After electrophoresis, remove gel from cassette(s) and briefly place in a tray containing deionized water or transfer buffer. This will ensure even wetting, facilitate proper gel placement and improve contact with the membrane.

3. Assemble stack centered on the bottom half of the cassette (anode) as depicted in Figure 5.3. Use a blot roller to remove any trapped air bubbles. Removal of trapped air bubbles is essential for high-quality transfer. Two firm passes with the included blot roller are typically sufficient.

Note If assembling more than one stack on the anode surface, evenly space and center the sandwiches so the top cathode surface applies even pressure to the surface areas of the stack(s). Ensure there is a 1cm space around all stack edges to allow any gases to escape during transfer.

Note If assembling more than one stack on the anode surface, evenly space and center the sandwiches so the top cathode surface applies even pressure to the surface areas of the stack(s). Ensure there is a 1cm space around all stack edges to allow any gases to escape during transfer.

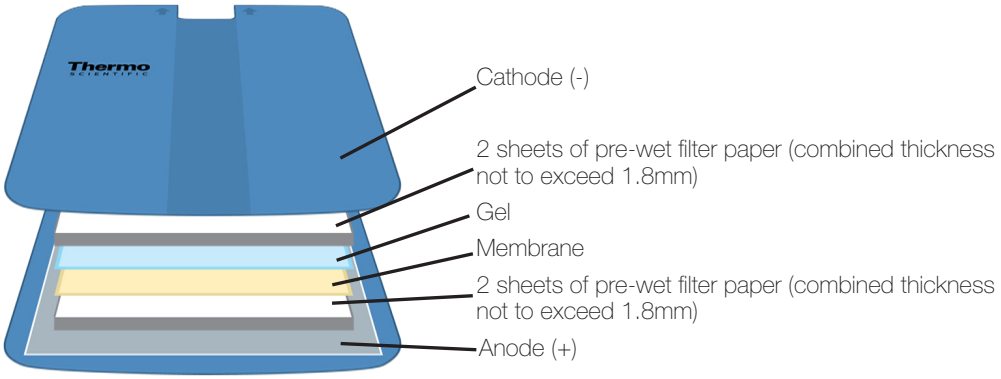
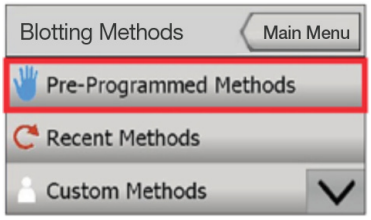


Figure 5.3. How to assemble power blot sandwich in the blot cassette.

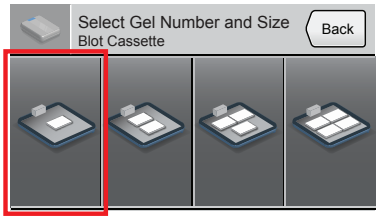
4. Gently press down top of cassette (cathode) to lock into place.

5. Slide cassette into the power station.

6. Select **Pre-Programmed Methods** in the **Main Menu**.



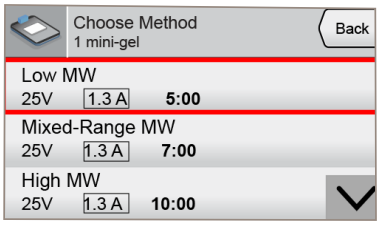
7. Using the touchpad, select the number of gels and gel size (mini or midi) you will transfer.



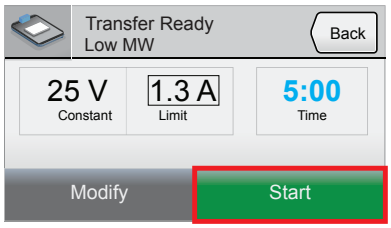
8. Choose the appropriate method to run (constant parameter in box):

a. Low MW (< 25kDa)	**25V**	**1.3A**	**5:00 min**
b. Mixed-Range MW (25-150kDa)	25V	1.3A	7:00 min
c. High MW (> 150kDa)	25V	1.3A	10:00 min
d. Std Semi-Dry	25V	1.0A	60:00 min
e. 1.5mm thick gels or unknown size gels	25V	1.3A	10:00 min

Note For fast-blotting programs (a, b, c and e), use Pierce 1-Step Transfer Buffer. Transfer time may be increased to 12 minutes for extremely high-molecular weight proteins or for slow-transferring gels. Do not use the Std Semi-Dry transfer program (d) with Pierce 1-Step Transfer Buffer.



9. Select the Start button to begin transfer.



10. Upon transfer completion, remove the transfer stack from the cassette(s) and thoroughly rinse the top and bottom section of the cassette.

Build-up of buffer salts will reduce cassette function and prevent the cassette from properly opening and closing. Rinse cassette(s) after every use.

WARNING After running at high current, the anode and cathode plates can become hot. Use caution when separating the gels and stacks from the plates. When continuously processing multiple samples at ~5A for a maximum of 2 hours, allow the cassette to cool for 30 minutes or use multiple cassettes to avoid excessive cassette heating.

Traditional Semi-Dry Transfer Method

Simultaneously transfer one to four mini-sized gels or one to two midi-sized gels in 45-60 minutes. Gels simultaneously transferred must have the same formulation.

1. Equilibrate gel(s) for 15 minutes in Towbin transfer buffer (25mM Tris, 192mM glycine, 20% methanol).

2. Cut two extra-thick (~2.48mm thick) sheets of Western blotting filter paper and one sheet of nitrocellulose or PVDF membrane to the same size as gel(s).

   Note The transfer stack should contain one sheet of ~2.48mm thick Western blotting filter paper on the bottom (anode), followed by membrane, gel and one sheet of ~2.48mm thick Western blotting paper on top. Do not exceed 2.48mm total filter paper thickness for the bottom of the stack or 2.48mm total filter paper thickness for the top of the stack. Use the appropriate Thermo Scientific filter paper:

   Product #88605, 2.48mm thick, 7.0cm x 8.4cm (mini-size)
   Product #88610, 2.48mm thick, 8.5cm x 9.0cm
   Product #88615, 2.48mm thick, 8.0cm x 13.5cm (midi-size)
   Product #88620, 2.48mm thick, 20.0cm x 20.0cm

3. Equilibrate filter paper and membrane in Towbin transfer buffer for at least 5 minutes.

   Note PVDF membrane must be wetted with methanol or ethanol before equilibration in Towbin transfer buffer.

   Note Filter paper and membrane stacks can be prepared ahead (few hours to overnight) and stored at 4°C.

4. Assemble stack on anode as depicted in Figure 5.4. Center stack(s) on anode to ensure even pressure. Use blot roller to remove any trapped air bubbles.

   Note Removal of trapped air bubbles is essential for high-quality transfer. Two firm passes with the included blot roller are typically sufficient.

   Note If assembling more than one stack on the anode surface, evenly space and center the sandwiches so the top cathode surface applies even pressure to the surface areas of the stack(s). Ensure there is a 1cm space around all stack edges to allow any gases to escape during transfer.

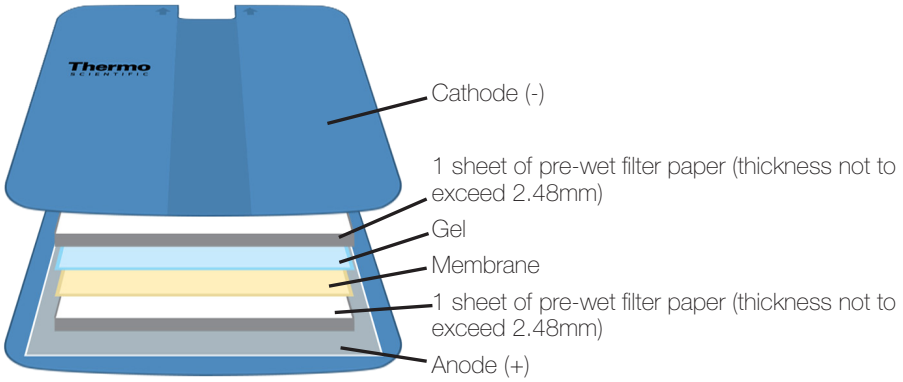
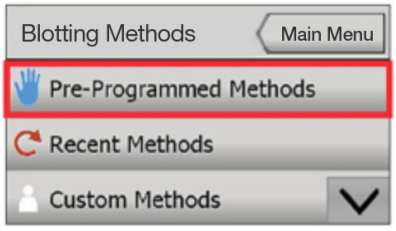


Figure 5.4. Orientation of filter paper, membrane and gel in transfer stack for standard semi-dry protocol.

5. Gently press down top of cassette (cathode) to lock into place. Slide cassette into the control unit.

6. Select **Pre-Programmed Methods** in the **Main Menu**.



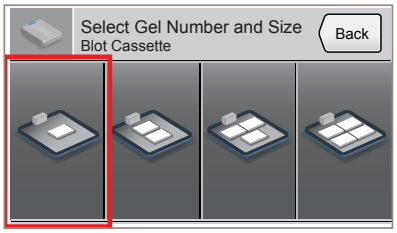
7. Select the number of gels and gel size (mini or midi) you wish to transfer:

   a. 1 mini-sized gel

   b. 2 mini-sized gels or 1 midi-sized gel

   c. 3 mini-sized gels

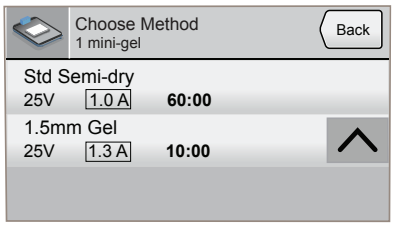
   d. 4 mini-sized gels or 2 midi-sized gels



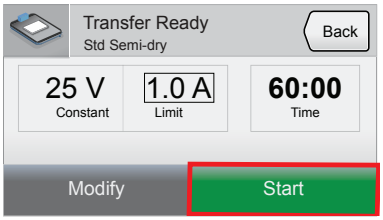
8. Choose program **Std Semi-dry** (constant parameter in box):

a.	Low MW (< 25kDa)	25V	1.3A	5:00 min
b.	Mixed Range MW (25-150kDa)	25V	1.3A	7:00 min
c.	High MW (> 150kDa)	25V	1.3A	10:00 min
d.	**Std Semi-dry**	**25V**	**1.0A**	**60:00 min**
e.	1.5mm thick or unknown size gels	25V	1.3A	10:00 min

Note For Std Semi-dry program (d), use Towbin transfer buffer or other conventional transfer buffer.



9. Select the **Start** button to begin transfer.



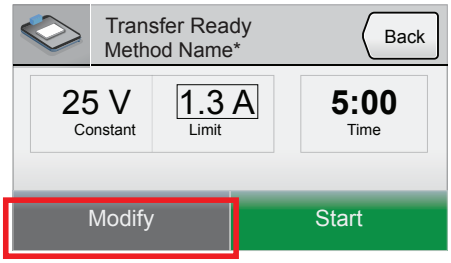
WARNING After running at high current, the anode and cathode plates can become hot. Use caution when separating the gels and stacks from the plates.

6

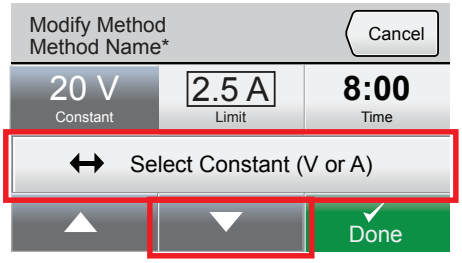
Chapter 6:
Custom Blotting Methods

Modifying Pre-Programmed Blotting Methods

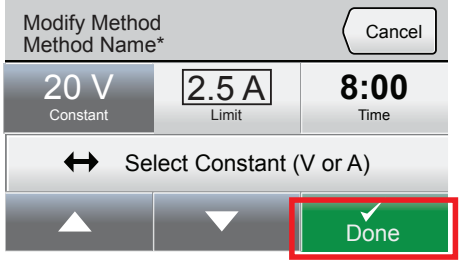
Pre-programmed methods can be modified before **Start** by selecting the **Modify** button.



1. Pressing the **Select Constant (V or A)** button will toggle the constant variable parameter from amps to volts or volts to amps.



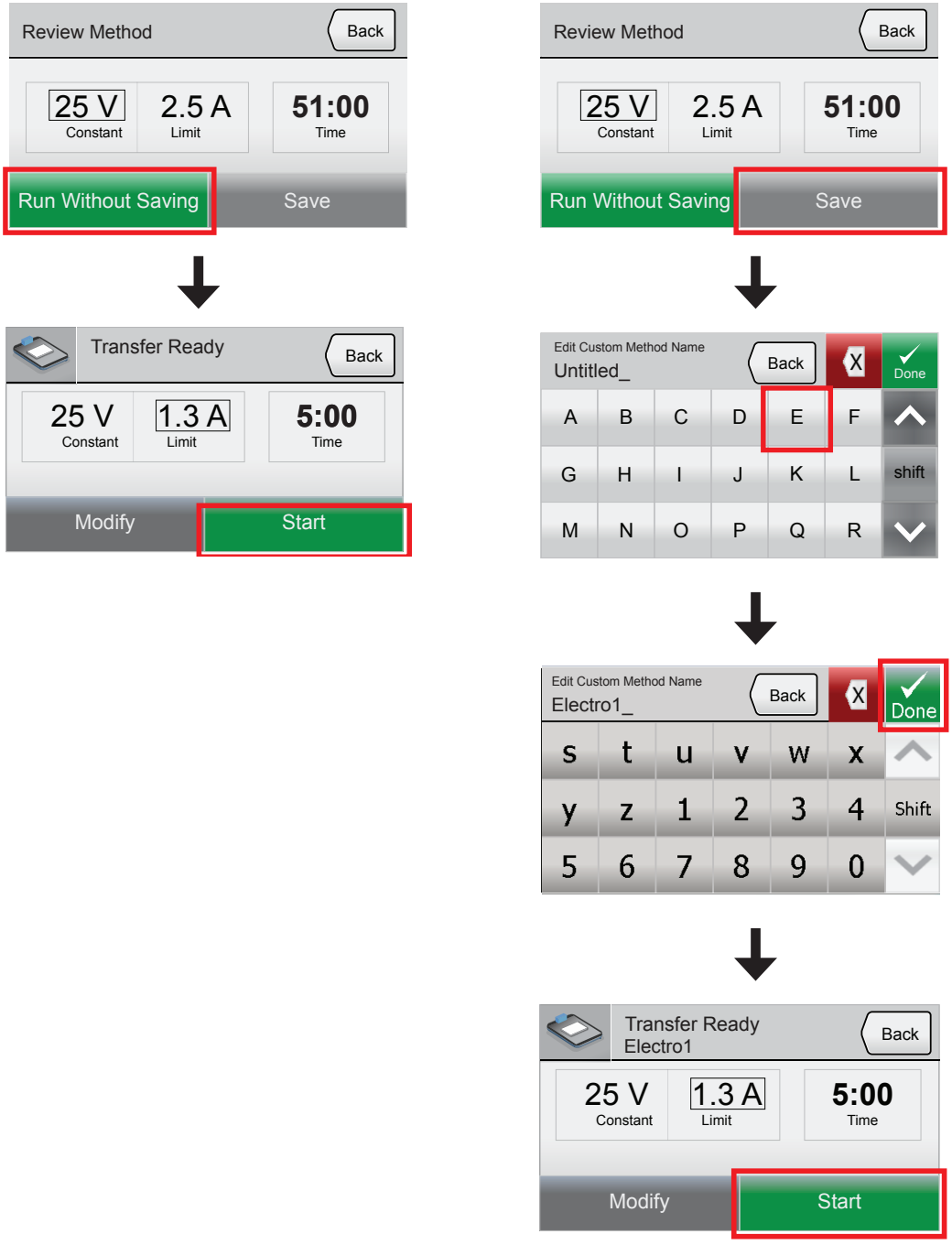
2. Highlight the variable for change and press the **Up** or **Down** arrow to raise or lower the selected variable's value.
3. After the program is modified, press the **Done** button.
4. The "Review Method" screen is now displayed. Press **Run Without Saving** or **Save**.



a. **Run Without Saving** will prompt the "Transfer Ready Screen." Press **Start** to begin the modified method.

b. If you wish to save the modified method, press **Save** and use the alphanumeric key pad to enter up to 15 characters to identify the new custom method. Press **Done** and then **Start** to begin the method. The custom method will be saved in **Custom Methods** in the **Main Menu**.

Creating a New Custom Method



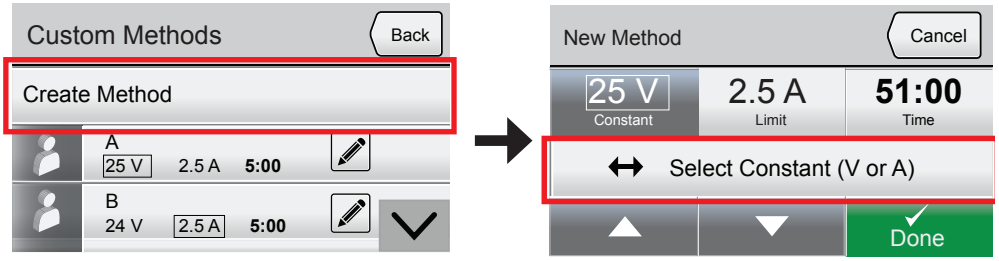
Custom methods are typically used when transferring non-standard gel sizes (i.e., not mini- or midi-sized). For rapid transfer protocols using Pierce 1-Step Transfer Buffer, measure the total surface area of the gel(s) you are transferring and multiply by 23mA/cm^2. For example, if transferring a 12cm x 15cm non-standard gel (measuring the gel and not the cassette), then use the following formula:

(12cm × 15cm × 23mA/cm^2) / 1000mA/A = 4.1A (constant)

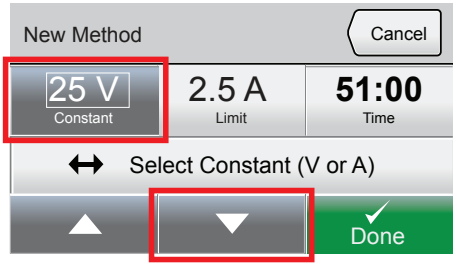
The voltage limit will be set at 25V. The amperage will be constant and set at 4.1A. The time will be set at 7 minutes for mid-range molecular weight proteins (25-150kDa).

Note Five amps is the maximum current setting allowed. If the gel area exceeds 220cm^2, set the amperage to 5A and compensate by increasing the transfer time by 1-2 minutes. For standard semi-dry protocols using Towbin buffer, voltage is held constant at 25V, amperage is limited to 1.0A and time is set to 30 minutes to 1 hour. Standard semi-dry programming does not take surface area into consideration.

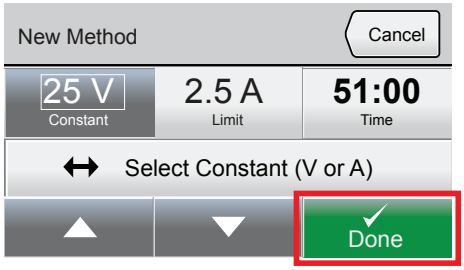
1. To create a custom method, press the **Custom Method** button on the **Main Menu**.
2. Select the **Create Method** button and press **Select Constant (V or A)** to toggle the constant variable parameter from amps to volts or volts to amps.



3. Highlight the variable for change and press the **Up** or **Down** arrow to raise or lower the selected variable's value.



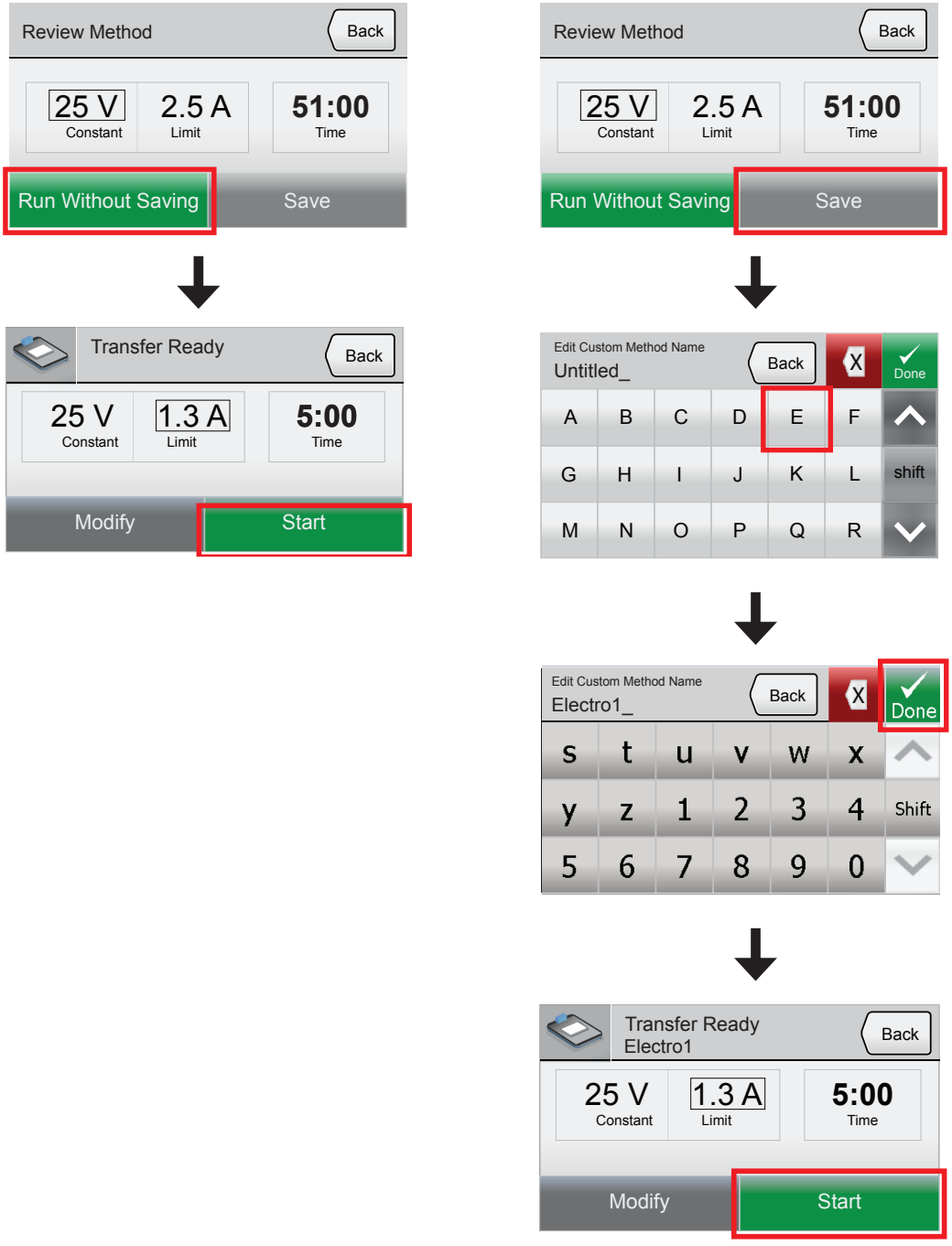
4. After the program is modified, press the **Done** button.



5. The Review Method screen is now displayed. Select **Run Without Saving** or **Save**.

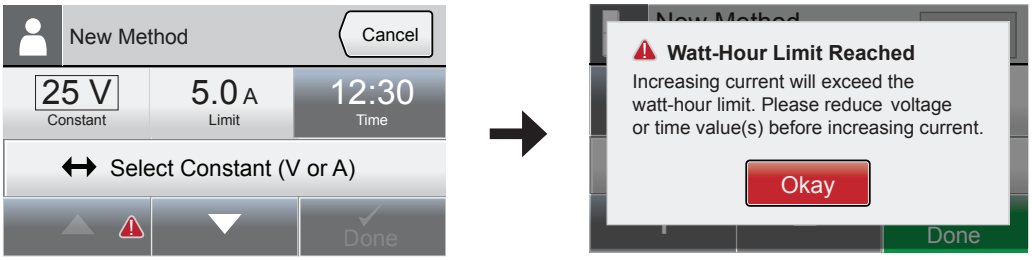
a. **Run Without Saving** will prompt the Transfer Ready screen. Press **Start** to begin the modified method.

b. If you wish to save the modified method, press **Save** and use the alphanumeric keypad to enter up to 15 characters to identify the new custom method. Press **Done** and then **Start** to begin the method. The custom method will be saved in Custom Methods in the **Main Menu**.



Recommended Maximum Watt-Hour Limit

To prevent damage to the cassette or control unit, custom methods are limited to **25 watt-hours** (when modifying pre-programmed methods and/or creating a new method). Any voltage or amperage adjustments that result in exceeding the maximum 25 watt-hours will bring up an alert triangle ⚠ on the **Up** arrow key. Continuing past this value will eventually result in a **Watt-Hour Limit** warning window.



The watt-hours are calculated using the formula Volts (V) x Amps (A) x Time (hours).

In the above example:

V = 25

A = 5.0

T = 12.5 / 60 (to convert to hours) = 0.21 (12:30 converted to 12.5 for calculation)

Watt-hours = 25V x 5.0A x 0.21 = 26.25

The 26.25 watt-hours exceed the 25 watt-hours limit, so the warning window appears.

If the **Watt-Hour Limit** warning window appears, select the **Okay** button to return to the previous screen and decrease the appropriate number (V, A or T) to return to a watt-hour value less than 25 watt-hours.

1. Pierce 1-Step Transfer Buffer is intended to rapidly transfer protein from gel to membrane using a high transfer current (based on transfer area) and a short time (5-10 minutes).
   a. Do not exceed 12-minute transfer times with rapid transfer protocols.
   b. Do not exceed 22-23mA/cm^2 (current/surface area) with rapid transfer protocols.
2. Conventional transfer buffers such as Towbin buffer (25mM Tris, 192mM glycine, 20% methanol) are low-ionic strength buffers and transfer time ranges from 30-60 minutes. Current normally spikes and then quickly drops to a very low level, while voltage reaches its maximum (25 volts).

Troubleshooting: Blotting

Problem	Possible Cause	Solution
All pre-stained molecular weight (MW) markers are not transferred out of the gel	Loading excess pre-stained MW markers (i.e., more than recommended by manufacturer) may result in a portion of the markers remaining in the gel after transfer	Use appropriate amount of pre-stained markers. It is normal for some protein to not transfer out of the gel, however sufficient proteins will be transferred for Western blotting. Even if some MW markers are left in the gel, proceed with the Western blot. Western blot sensitivity is the best indicator of transfer efficiency.
Poor transfer of high-MW proteins	Insufficient transfer time or inappropriate gel type used (i.e., 4-20% Tris-Glycine gradient gels are not recommended for proteins > 200kDa	Increase transfer time using High MW Pre-Programmed Method (10-12 minutes). Use appropriate gel type and percentage for electrophoresis of high-molecular weight proteins. Use low-percentage, non-gradient polyacrylamide gels (4%, 6%, or 8% Tris-Glycine gels) or 3-8% gradient or 7% non-gradient Tris-Acetate gel.
Cassette is difficult to open	Salt deposited on moving parts inside cassette	Rinse or immerse the unassembled cassette top and bottom in warm water while removing any sticky salt residue with a gloved hand. Rinse with deionized water and place perpendicular in a rack to dry. **NOTE: Failure to keep cassette top (cathode) and bottom (anode) clean will result in the moving parts sticking, leading to poor transfer efficiency**
Inconsistent transfer	Membrane or filter paper insufficiently equilibrated in Pierce 1-Step Transfer Buffer	Equilibrate membrane and filter paper in Pierce 1-Step Transfer Buffer before transfer for a minimum of 10 minutes. Use a sufficient amount of buffer for the equilibration step
	Incorrect transfer buffer used	Use Pierce 1-Step Transfer Buffer for rapid transfer
	Insufficient transfer time	Increase transfer time from 5-10 minutes to 7-12 minutes
	Filter paper and membrane are not cut to same size as gel	Cut the filter paper and membrane to the same size as the gel. Ensure there is no overhang around the sides of the transfer stack
	PVDF membrane was not pre-wetted with methanol or ethanol	Wet PVDF membrane with methanol or ethanol and equilibrate for 10-15 minutes in Pierce 1-Step Transfer Buffer before transfer
	Air bubbles trapped between gel and membrane	When assembling transfer stack, use a roller or pipette to remove any air bubbles between the gel and the membrane
	Filter paper used in the fast transfer exceeded 1.8mm thickness	Use filter paper < 1.8mm thick
Inefficient transfer of low-MW proteins to PVDF	Inefficient binding of some low-MW proteins (< 25kDa) to PVDF membrane	Combine ethanol and Pierce 1-Step Transfer Buffer in a 15:85 ratio before equilibrating filter paper and membrane (e.g., 15mL of ethanol with 85mL of Pierce 1-Step Transfer Buffer)

7

Chapter 7: Maintenance

Cleaning the Pierce Power Station LCD Touchscreen

IMPORTANT Use of ammonia-based cleaners to clean the touchscreen is NOT recommended. Do not spray cleaner directly onto the LCD screen.

IMPORTANT Do not press hard while cleaning the screen. Do not leave excess cleaning solution on the LCD screen as this may cause long-term damage.

1. Use a pre-moistened LCD screen-cleaning tissue to clean the surface of the touchscreen.

Cleaning the Pierce Power Station Housing and Cassette

IMPORTANT When using cleaning products with the stainer, consult the appropriate safety data sheet (MSDS) to confirm compatibility and proper usage of the cleaning product.

1. Spray a soft cloth or paper towel with cleaning solution (e.g., general-purpose cleaner containing water and mild detergent, isopropanol, or 70% ethanol). Avoid abrasives and organic solvents.
2. Wipe the exterior surfaces of the control unit and cassette. Use caution to avoid touching the LCD touchscreen with anything other than a screen-cleaning wipe.
3. Ensure that the cooling fan vent is free of dust and debris.

Cleaning the Anode and Cathode Plates

IMPORTANT Always wear gloves when touching the anode and/or cathode.

1. After each use, thoroughly rinse the anode and cathode under warm water.
2. Rinse with deionized water and stand parts in a rack to dry.

thermofisher.com/techresources

International Customers
Go to www.lifetech.com/ordersupport and select your specific location from the drop-down list at the bottom of the page. Selecting your country will provide contact and technical service details for local distributors.

United States
Tel: 815-968-0747 or 800-955-6288
Customer Assistance E-mail:
customerservice@lifetech.com
thermofisher.com/techresources

Thermo SCIENTIFIC
A Thermo Fisher Scientific Brand

2162585.1 29 January 2016