

Technical Tip 09

ZEUS AtheNA Multi-Lyte® Test Systems

Subject: BioTek (Model: ELx 50) Filter Plate Washer Adjustment Procedure

BioTek filter plate washers may require adjustments in order to compensate for wear and filter plate variability. The optimization of wash aspiration pressure and length of aspiration time will ensure optimal AtheNA Multi-Lyte[®] read times. Please follow the procedure below to verify and make necessary adjustments as recommended for optimal filter plate vacuum wash:

- 1. Set the BioTek washer to medium. This is done by ensuring that the opening under the release lever, at the front of the plate holder, contains a grey plug.
- 2. Create new (10 second) first aspiration program using the washer keypad (Refer to BioTek ELx50 Washer Manual if needed):
 - a. Select Main Menu
 - b. Select Define
 - c. Select Create
 - d. Select More
 - e. Select Aspir
 - f. At Enter Name, you may free text the name of new program. However, for consistency, it is recommended to name it as "BOT10". Hit Enter.
 - g. At Type, enter BOT. Hit Enter.
 - h. At Aspiration Time, enter 010 (for 10 seconds). Hit Enter.
 - i. Return to Main Menu
- 3. Create new (18 second) subsequent aspirations program using the washer keypad (Refer to BioTek Washer Manual if needed):
 - a. Select Main Menu
 - b. Select Define
 - c. Select Create
 - d. Select More
 - e. Select Aspir
 - f. At Enter Name, you may free text the name of new program. However, for consistency, it is recommended to name it as "BOT18". Hit Enter.
 - g. At Type, enter BOT. Hit Enter.
 - h. At Aspiration Time, enter 018 (for 18 seconds). Hit Enter.
 - i. Return to Main Menu



- 4. Create a link for the new wash program using the keypad (Refer to BioTek Washer Manual if needed):
 - a. At Main Menu, select Define
 - b. Select Create
 - c. Select More
 - d. Select Link
 - e. At enter Name, you may free text the name of new link. However, for consistency, it is recommended to name it as "AT10". Hit Enter.
 - f. Select More
 - g. Select Aspir
 - i. Select Aspir Program by scrolling through those that are available to locate new first aspiration program created in step #2 named "BOT10". In order to scroll, use the keypad and type 01, then 02, then 03, then 04 etc. until "BOT10" appears. Once located, hit Enter.
 - h. At Select Link 02 Type, select Disp
 - Select Disp Program (which is already preprogrammed and will not change) by scrolling until "ATHENADISP" is located. In order to scroll, use the keypad and type 01, then 02, then 03, then 04 etc. until
 - "ATHENADISP" appears. Once located, hit Enter.
 - i. At Select Link 03 Type, select More.
 - j. Select Asp
 - i. Select Aspir Program by scrolling through those that are available to locate new subsequent aspirations program created in step #3 named "BOT18". In order to scroll, use the keypad and type 01, then 02, then 03, then 04 etc. until "BOT18" appears. Once located, hit Enter.
 - k. At Select Link 04 Type, select Disp
 - i. Select Disp Program (which is already preprogrammed and will not change) by scrolling until "ATHENADISP" is located.
 - In order to scroll, use the keypad and type 01, then 02, then 03, then 04 etc. until "ATHENADISP" appears. Once located, hit Enter.
 - I. At Select Link 05 Type, select More.
 - m. Select Asp
 - i. Select Aspir Program by scrolling through those that are available to locate new subsequent aspirations program created in step #3 named "BOT18". In order to scroll, use the keypad and type 01, then 02, then 03, then 04 etc. until "BOT18" appears. Once located, hit Enter.
 - n. At Select Link 06 Type, select Disp
 - i. Select Disp Program (which is already preprogrammed and will not change) by scrolling until "ATHENADISP" is located.
 - In order to scroll, use the keypad and type 01, then 02, then 03, then 04 etc. until "ATHENADISP" appears. Once located, hit Enter.
 - o. At Select Link 07 Type, select More.



- p. Select Asp
 - i. Select Aspir Program by scrolling through those that are available to locate new subsequent aspirations program created in step #3 named "BOT18". In order to scroll, use the keypad and type 01, then 02, then 03, then 04 etc. until "BOT18" appears. Once located, hit Enter.
- q. At Select Link 08, return to the Main Menu. At "OK to Save Program?", select Yes.

Note: There are a total of 7 steps (links) which account for the entire washing procedure: asp, disp, asp, disp, asp. The dispense portion of the link/ program will not change. However, it is still necessary to build an entire link to include them with the newly created aspiration time program as indicated above.

- 5. Fill all of the wells of an unused filter plate with 60 microliters of sample diluent. Place the filter plate onto the washer. Select RUN from the Main Menu.
- 6. Select More.
- 7. Select Link.
- 8. Select Link
 - i. Scroll using the keypad, until the new program "AT5" that was created in step #3, e, is located. In order to scroll, use the keypad and type 01, then 02, then 03, then 04 etc. until "AT5" appears. Once located, hit Enter.
- 9. Enter "12" as the total number of strips to be washed. Hit Enter.
- 10. Select Start.
 - a. While the plate washing occurs, observe that all of the wells of the entire plate are evacuated completely during each aspiration step. There should be no fluid remaining in the wells BEFORE the subsequent dispense step. The initial sample diluent solution WILL BE SUFFICIENTLY ASPIRATED since only 60 microliters of solution was pipetted. However, it is important to carefully observe the subsequent aspirations since these will contain 200 microliters of dispensed wash buffer.
 - b. Upon completion of the entire plate washing process, remove the plate and verify that the wells are completely empty. If they are not and fluid was not sufficiently aspirated between dispense steps, then another wash program utilizing a 7 second aspiration time must be created beginning with Step #2, but substituting the number 7 in all programs as indicated below:
- 11. Select Main Menu
 - a. Select Define
 - b. Select Create
 - c. Select More
 - d. Select Aspir
 - e. At Enter Name, you may free text the name of new program. However, for consistency, it is recommended to name it as "BOT7". Hit Enter.
 - f. At Type, enter BOT. Hit Enter.
 - g. At Aspiration Time, enter 007 (for 7 seconds). Hit Enter.
 - h. Return to Main Menu



- 12. Create a link for the new wash program using the keypad (Refer to BioTek Washer Manual if needed):
 - a. At Main Menu, select Define
 - b. Select Create
 - c. Select More
 - d. Select Link
 - e. At enter Name, you may free text the name of new link. However, for consistency, it is recommended to name it as "AT7". Hit Enter.
 - f. Select More
 - g. Select Aspir
 - i. Select Aspir Program by scrolling through those that are available to locate new one created in step #2 named "BOT7". In order to scroll, use the keypad and type 01, then 02, then 03, then 04 etc. until "BOT7" appears. Once located, hit Enter.
 - h. At Select Link 02 Type, select Disp
 - Select Disp Program (which is already preprogrammed and will not change) by scrolling until "ATHENADISP" is located.
 In order to scroll, use the keypad and type 01, then 02, then 03, then 04 etc. until

"ATHENADISP" appears. Once located, hit Enter.

- i. At Select Link 03 Type, select More.
- j. Select Asp
 - i. Select Aspir Program by scrolling through those that are available to locate new one created in step #2 named "BOT5". In order to scroll, use the keypad and type 01, then 02, then 03, then 04 etc. until "BOT7" appears. Once located, hit Enter.
- k. At Select Link 04 Type, select Disp
 - Select Disp Program (which is already preprogrammed and will not change) by scrolling until "ATHENADISP" is located.
 In order to scroll, use the keypad and type 01, then 02, then 03, then 04 etc. until "ATHENADISP" appears. Once located, hit Enter.
- I. At Select Link 05 Type, select More.
- m. Select Asp
 - i. Select Aspir Program by scrolling through those that are available to locate new one created in step #2 named "BOT5". In order to scroll, use the keypad and type 01, then 02, then 03, then 04 etc. until "BOT7" appears. Once located, hit Enter.
- n. At Select Link 06 Type, select Disp
 - Select Disp Program (which is already preprogrammed and will not change) by scrolling until "ATHENADISP" is located.
 In order to scroll, use the keypad and type 01, then 02, then 03, then 04 etc. until

"ATHENADISP" appears. Once located, hit Enter.

- o. At Select Link 07 Type, select More.
- p. Select Asp



- i. Select Aspir Program by scrolling through those that are available to locate new one created in step #2 named "BOT7". In order to scroll, use the keypad and type 01, then 02, then 03, then 04 etc. until "BOT7" appears. Once located, hit Enter.
- q. At Select Link 08, return to the Main Menu. At "OK to Save Program?", select Yes.
- 13. Fill all of the wells of an unused filter plate with 60 microliters of sample diluent. Place the filter plate onto the washer. Select RUN from the Main Menu.
- 14. Select More.
- 15. Select Link.
- 16. Select Link
 - i. Scroll using the keypad, until the new program "AT7" that was created in step #3, e, is located. In order to scroll, use the keypad and type 01, then 02, then 03, then 04 etc. until "AT7" appears. Once located, hit Enter.
- 17. Enter "12" as the total number of strips to be washed. Hit Enter.
- 18. Select Start.
 - a. While the plate washing occurs, observe that all of the wells of the entire plate are evacuated completely during each aspiration step. There should be no fluid remaining in the wells BEFORE the subsequent dispense step. The initial sample diluent solution WILL BE SUFFICIENTLY ASPIRATED since only 60 microliters of solution was pipetted. However, it is important to carefully observe the subsequent aspirations since these will contain 200 microliters of dispensed wash buffer.
 - b. Upon completion of the entire plate washing process, remove the plate and verify that the wells are completely empty. If they are not and fluid was not sufficiently aspirated between dispense steps, then another wash program utilizing a 8 second aspiration time must be created beginning with Step #2, but substituting the number 8 in all programs as indicated. Continue this process until an optimum aspiration time has been determined.