

PROTOCOL: Protein Level Normalization (DIL)

Purpose

To create a plate with variable amounts of excess lysis buffer to be mixed with a uniform amount of sample in order to normalize protein concentration across samples.

Preparation

1. Prepare Bender for use by purging and flushing the lines.
2. Place all labware (tips, plates, reagents, etc...) on the deck.

Materials

- P100 Lysis Buffer {DIL-M01} - from LYSIS protocol
- 96-Well 2.2mL Deep Well plate, VWR, Cat. No. 82006-448 {DIL-M02}
- 900uL Conductive Tips with Filter, Perkin Elmer, Cat. No. 6001256 {DIL-M03}
- 175uL Conductive Tips with Filter, Perkin Elmer, Cat. No. 6000687 {DIL-M04}
- 50uL Conductive Tips, Perkin Elmer, Cat. No. 6001264 {DIL-M05}
- Reagent Trough (double 25mL), Perkin Elmer, Cat. No. 6000662 {DIL-M06}

Assets

- Perkin Elmer JANUS (Bender) {DIL-A01}

Reagent Mixes

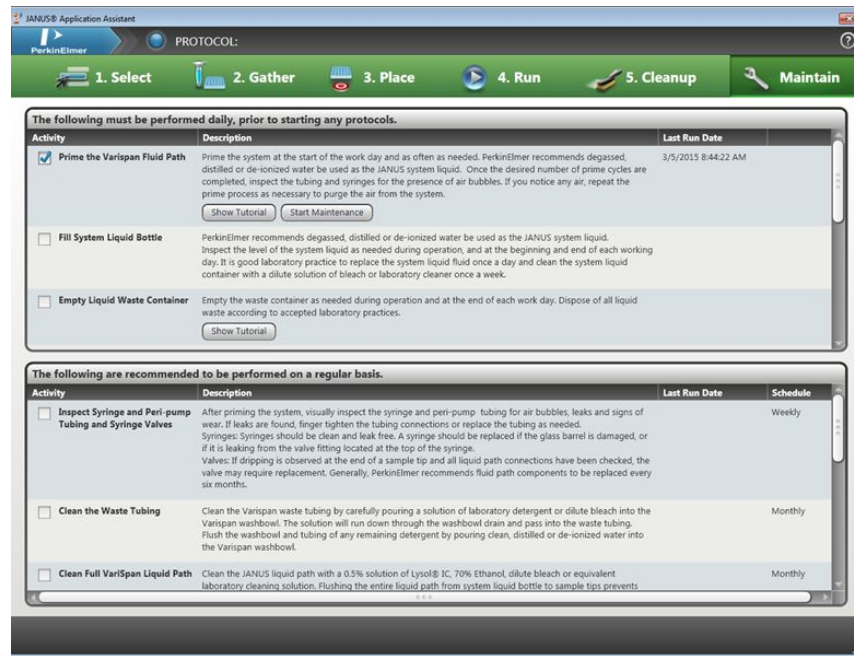
N/A

Procedure

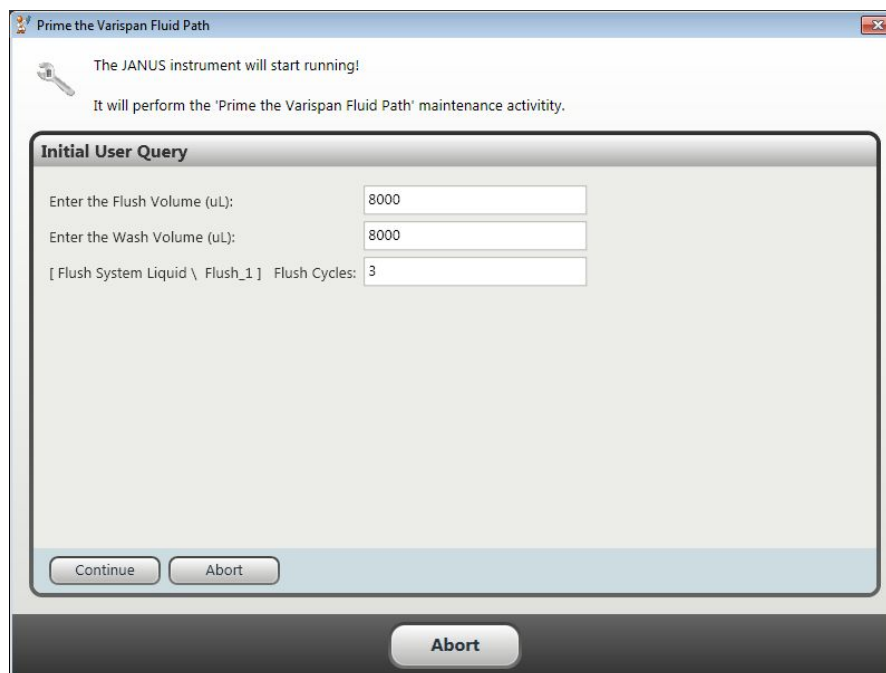
1. Purge the system fluid on Bender {DIL-A01} by opening Janus Application Assistant on the desktop:



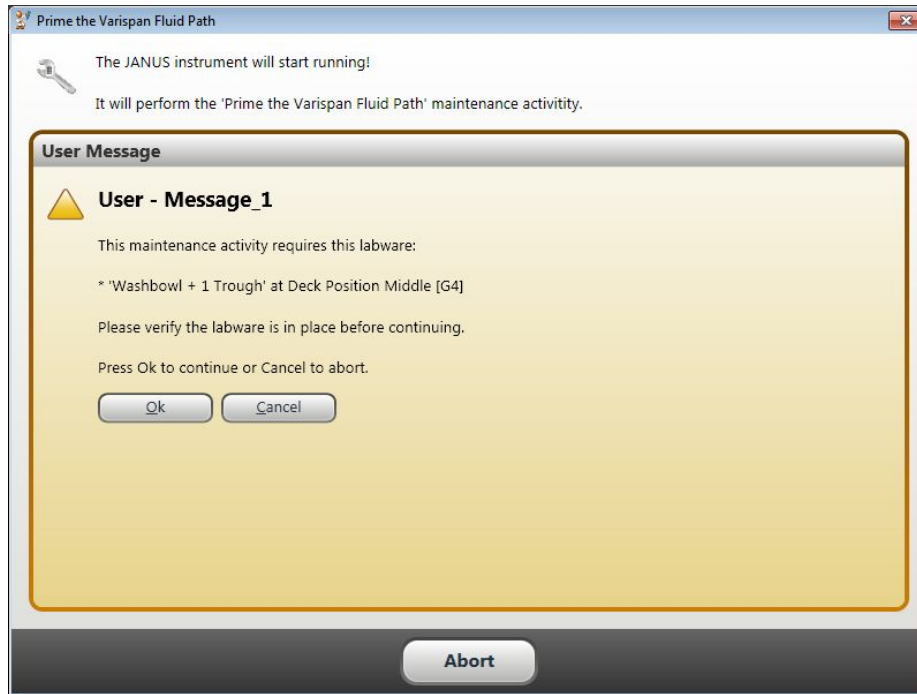
2. When the application opens, click on Maintain and check the box titled "Prime the Varispan Fluid Path". Click on "Start Maintenance".



3. Input the Flush and Wash volume as 8000uL and the number of cycles to 3.



4. Press continue and the following screen will come up:

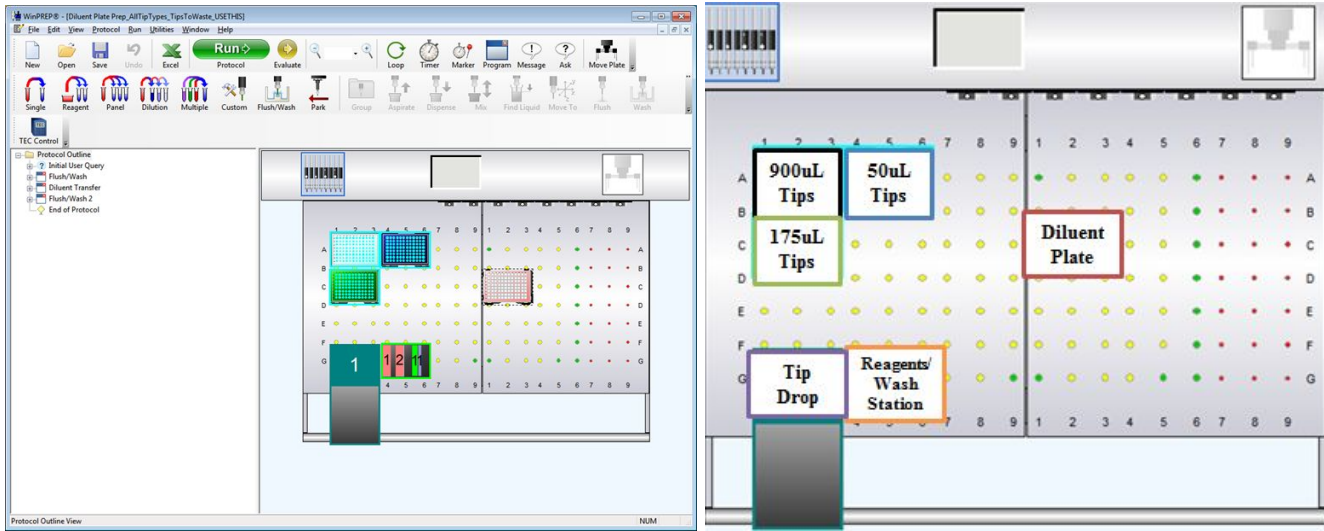


5. Press ok to run the startup protocol.
6. When the protocol is complete, press ok on the popup and the Janus Application Assistant will close.
7. Open WinPREP for JANUS:

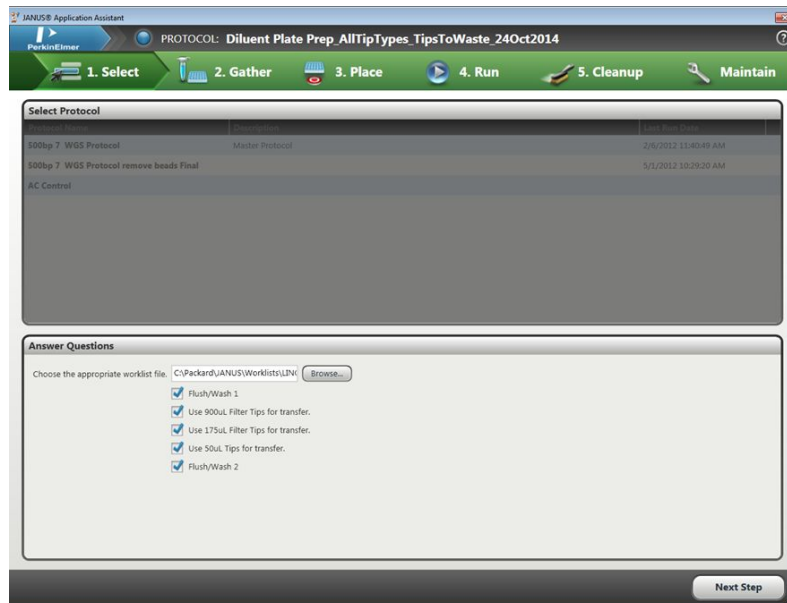


8. Open the protocol file "Diluent Plate Prep_AllTipTypes_TipsToWaste_USETHIS" found at C:\\Packard\\JANUS\\Protocol Files\\.

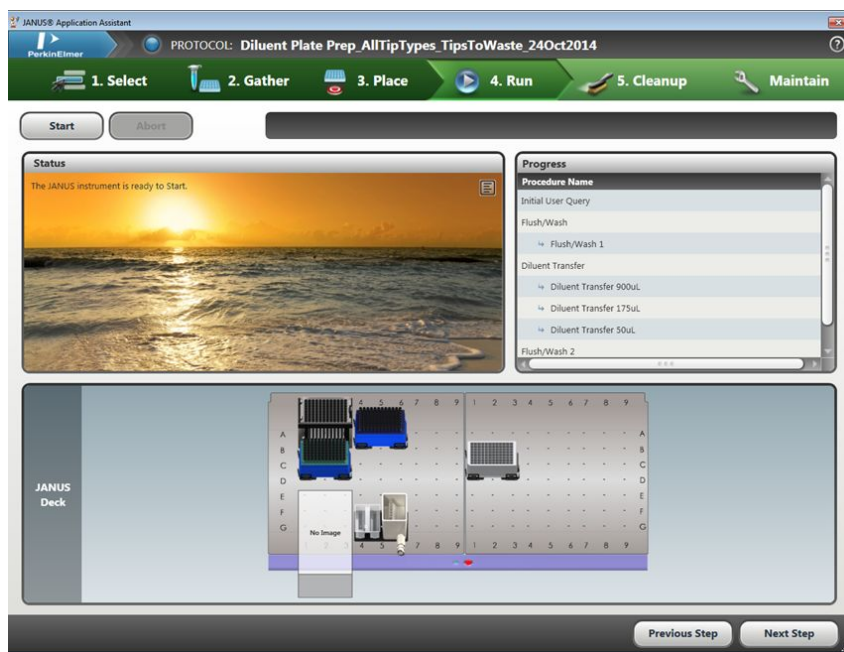
9. When the file is open, the screen will display how the deck should be setup. Press “Run” to start the protocol.



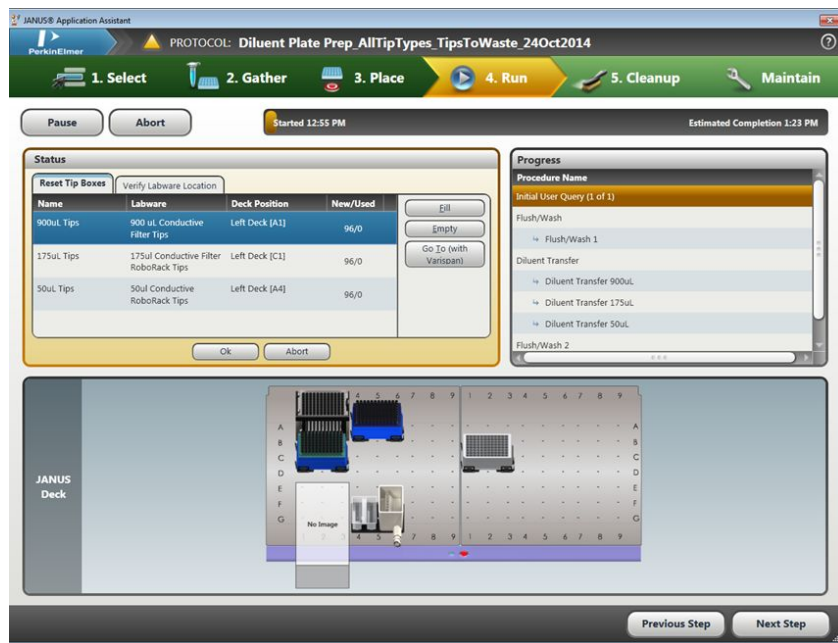
10. You can check or uncheck which portions of the protocol to run. Browse to choose the appropriate worklist file which will be created when you calculate the amounts of diluent to deliver.



11. Press Run at the top of the screen.



12. Ensure that all tip boxes are filled, then press ok to run the protocol.



13. When the protocol is complete:

13.1. Clear the deck.

13.2. Continue on to transfer sample lysate to the newly created **Diluent Plate**.