





## **Autodiluter Count Standard Procedure: READ INSTRUCTIONS BEFORE TESTING**

This procedure is written for the use of this Count Standard on the 780 AccuSizer Autodiluter. Refer to the documentation/instructions of your system for additional operating instructions.

1. Turn instrument on and allow the instrument to warm up for ½ hour.
2. Start software.
3. Verify that the reported sensor voltage (see the sensor status screen from the View pull down menu) is within 10% of the maximum observed voltage. If not remove sensor and clean.
4. Read the menu file labeled “contstd1.rcp”. The menu file is included on diskette and can be copied into the PSS software directory or can be read from the diskette.  
Note: If the diskette contains no information or is unreadable refer to the Control Setup screen capture included and copy the operating parameters.
5. Set the Data Directory and File Name by clicking on <Browse> from the Control Setup menu that appears and click <OK>.  
Note: at this time the user may choose to save the new menu file onto their computer hard drive for easy access in the future.
6. Set the minimum detected size of the instrument to 0.56 um (lower threshold is set by using the up and down cursor keys when the screen is blank and can be observed in right most box of the status bar).
7. Begin a flush of the instrument using the  button on the tool bar.
8. When flushing verify flow rate of instrument using a graduated cylinder on the drain line. Flow should be 60ml/min. To adjust the flow rate increase or decrease the “flow pump factor” in the Setup menu accordingly until 60ml/min flow rate is verified on the drain line with the graduated cylinder.
9. Stop the stir bar
10. Drain the dilution flask until the fluid level is at the bottom of the vessel . NOTE: do NOT allow the diluent to drain out the stem between the flask and the sensor but DO allow the maximum amount of liquid to drain from the system.
11. Fill the dilution flask using the  button on the tool bar. You may notice there is less diluent in the flask than you normally use, this test is designed to use 25 ml of diluent.
12. Start the stirring of the dilution flask contents at a rate approximating 120 rpm (if using a 780 system with stir bar control, set the normal speed factor to 1400 and make sure the Stir Speed Control box is checked).
13. Begin a flush of the instrument using the  button on the tool bar. The system is clean and ready for the test when the user observes 4 consecutive counts of 10#/ml or less on the status bar.
14. Prepare a pipettor and tips. Choose a pipettor that can dispense 100-500ul of liquid sample.
15. Prepare the count standard for immediate injection into the dilution flask
  - a. mix the bottle by inverting 25 times

- b.       sonicate the sample by placing the bottle in a sonic bath for 30 seconds  
**NOTE:** if the sample rests for more than 60 seconds before pipette extraction prepare the sample again.
16. Extract aliquot from sample bottle, replace bottle cap, and immediately dispense into the dilution flask.  
**NOTE:** sample should be dispensed from the pipettor at a slow rate below the surface of the diluent if possible. Counts are easily introduced into the measurement by aerating the sample or diluent. If possible, after dispensing sample, pull up diluent into pipette tip and dispense back into flask (slowly) to rinse pipette tip.
17. Click on the Start measurement from the tool bar.
18. Ensure the Data file is saved to the computer for analysis.

### Analyzing the Data

19. Read the data file just created
20. Select the user defined peak from the display pull down menu. A window will appear at the bottom of the screen displaying data on the full range of the distribution.
21. Place the mouse cursor over the graph and click. This produces a cross hair on the graph, use the cursor <left> and <right> keys on the keyboard to move the cross hair to the first Lower Channel Size as defined by the attached Certificate of Analysis and press <Enter>. Position of the cross hair is displayed on the status bar.
22. Then move the cross hair to the Upper Channel Size as defined by the attached Certificate of Analysis and press <Enter>.
23. The software places a vertical black line on each side of the peak indicating the range of the sizes you wish to examine. The window at the bottom of the screen now contains information about this range. Ensure the range is correct and record the “mean diameter” and the “# of Particles in Range”. Repeat this for each peak in the count standard.
24. The software allows 3 User Defined Peaks to be defined and can be printed by choosing “Cumulative Table” from the print menu.
25. To calculate the concentration of particles in #/ml in each peak, multiply the # of particles in range by the dilution factor and divide by the aliquot size in ml.  
E.G. (# of particles = 76983 X Dilution Factor 1.31) / 0.100 ml injection. Results in 1,008,477#/ml
26. Compare the mean and total counts obtained for each peak with the expected results and range as defined by the Certificate of Analysis. The mean size should agree within 5% and counts should fall within the defined range.

The number of particles reported by your AccuSizer 780 Autodiluter should fall within the ranges defined on the Certificate of Analysis provided that the sample was prepared in accordance with the instructions provided and the instrument has been adjusted properly for sample flow rate and cleanliness. If your results do not fall within the range defined by the Certificate of Analysis please check these parameters and repeat the test.

Do not rely on the exact counts in the bottle after the liquid level has reached below 50% of the original bottle volume as the statistical confidence of the counts will be below acceptable values.

The nature of sampling is by definition a statistical process. Factors such as stirring speed, pipette tip location, sample volume and flow rate of pipettor have an influence on the spatial distribution of the particles within the bottle, which determines the particles that are actually sampled/counted.

## Menu Parameters

**Control Setup** [X]

Menu File: C:\PSS Software\CW788\countstd.rcp

Data Directory: C:\data\02 data\count standard\ Browse

File Name: 040402.700

Caption: cnt standard Lot 3A02154

Collection Time: 60 Sec.

Number Channels: 512

Vessel Fluid Volume: 25 ml

Flow Rate: 60 ml/min

Max Coincidence: 6000 #/ml

No. Autocycles: 1

Sample Calculation:  
 Mass Fraction  PPM/PPB  
 Volume Fraction  None

Sample Flow Time: 10 Sec

Number of Samples: 1

Sampling Period: 2 Sec

Automatic Data Save  Save ASCII File Browse

Overwrite Old File

Automatic Flush

File Name: C:\test.asc

Subtract Background

Background File: NONE Sel. Background File

OK Cancel