Sample Retention Pedestal Measurements

A 1 - 2 µL sample is pipetted onto a measurement pedestal. A smaller, 0.5 µL volume sample, may be used for concentrated nucleic acid and protein A280 samples. A fiber optic cable (the receiving fiber) is embedded within this pedestal. A second fiber optic cable (the source fiber) is then brought into contact with the liquid sample causing the liquid to bridge the gap between the ends of the two fibers. A pulsed xenon flash lamp provides the light source and a spectrometer utilizing a linear CCD array analyzes the light passing through the sample. The instrument is controlled by PC based software, and the data is stored in workbook files (*.twbk) on the PC.

Pedestal Sample Size Requirements

Although sample size is not critical, it is essential that a liquid column is formed when using the pedestal option so that the pathlength between the upper and lower measurement pedestals is bridged with sample.

The dominant factor determining the surface tension of a droplet is the hydrogen bonding of the lattice of water molecules in solution. Generally, all additives (including protein, DNA, RNA, buffer salts and detergent-like molecules) can reduce the surface tension by interfering with the hydrogen bonding between water molecules. Although 1 µL volumes are usually sufficient for most sample measurements, increasing the sample size to 2 µL will ensure proper column formation for samples with reduced surface tension.

Field experience indicates that the following volumes are sufficient to ensure reproducibility:
Aqueous solutions of nucleic acids: 1 µL
Purified protein: 2 µL
Bradford, BCA, Lowry or Protein Pierce 660 nm assays: 2 µL
Microbial cell suspensions: 2 µL

It is best to use a precision pipettor (0-2 µL) with precision tips to ensure that sufficient sample (1-2 µL) is delivered. Lower precision pipettors (0-10 µL and larger) are not as good at delivering 1 µL volumes to the measurement pedestal. If the user is unsure about the sample characteristics or pipettor accuracy, a 2 µL sample volume is recommended.

Pedestal Basic Use

1. Raise the sampling arm and pipette the sample onto the lower measurement pedestal.

2. Lower the sampling arm and initiate a spectral measurement using the software on the PC. The sample column is automatically drawn between the upper and lower pedestals and the measurement is made.

3. When the measurement is complete, raise the sampling arm and wipe the sample from both the upper and lower pedestals using a dry, lint-free laboratory wipe. Simple wiping prevents sample carryover in subsequent measurements for samples varying by more than 1000 fold in concentration.