Malvern Zetasizer Nano User Manual

Sample Preparation

Size Measurements

For size measurements, particle size and sample concentration must be taken into consideration. Follow the below guidelines for optimal measurements:

- Sample concentration depends on the sample material
  - If the concentration is too low, then there might not be enough light scattering for the measurement. If the concentration is too high, then light scattering from one particle might scatter again by another particle.
  - **Maximum concentration is when the sample can no longer freely diffuse** due to particle interactions. This is primarily dictated by the particle size. Below is a chart that can approximate the maximum and minimum concentrations for different particle sizes.

Particle Size	Recommended Minimum Concentration	Recommended Maximum Concentration
< 10 nm	0.5 mg/ml	Only limited by the sample material interaction, aggregation, gelation etc.
10 nm to 100 nm	0.1 mg/ml	5% mass (assuming 1g/cm^3 density)
100 nm to 1 mm	0.01 mg/ml (10^{-3} % mass)	1% mass (assuming 1g/cm^3 density)
> 1 mm	0.1 mg/ml (10^{-2} % mass)	1% mass (assuming 1g/cm^3 density)

* Approximate values based on the particles having a reasonable difference in refractive index to that of the dispersant.

- Select the sample concentration where the **solution creates a milky/turbid appearance**.
- **Filter all liquids used to dilute the sample** (dispersants and solvents). Filter size will depend on the estimated sizes of the particles. This is to remove any dust or contaminants in the solution.
- **Ultrasonication can also be used to remove air bubbles or break up agglomerates**, but care must be taken so that primary particles are not damaged.

Molecular Weight Measurements

Molecular weight measurement follows similar protocols as size measurements, but with more aspects to be considered since the techniques is very sensitive.

- All solvents must be filtered to 0.02 µm or better.
- The prepared solutions should be allowed to stand for a period, depending on the sample, to ensure adequate solvation.
- All glassware and apparatus must be cleaned and free of scratches and dust.
- Very small samples such as proteins in aqueous solutions will often require filtering.

The molecular weight measurement requires a series of diluted samples with concentrations typically between 0.25 to 1 gl^{-1}. The samples must be fully soluble and dust free. From this, a Deby plot can be generated by measuring the scattering of light by the solvent and samples of various concentrations.

- Minimum concentration that should be used is defined by the excess scattering over the solvent which should be a minimum of 30%.

- Maximum concentration is dependent upon the onset of particle interactions. Typically keep the maximum concentration below 0.1 w/v%.

Zeta Potential Measurements

Concentration requirements are not as strict for zeta potential measurements compared to size and molecular weight studies because of the optical arrangement. There is an auto attenuator to ensure that the sample count rate (see below) is suitable for the requirements of the detector. However, **if the concentration is too high then a low sample count rate error will be displayed**. **If too low, the attenuator will be maxed out and the results will be variable**. Also, if there is a bubble in the sample cell, a low sample concentration error will be shown.

Sample dilution will be required and critical for determining the final value measured. The dilution medium is very important, and the **zeta potential is dependent on the composition of the disperse phase since it is on the nature of the particle surface**.

Careful selection must be made to preserve the existing state of the surface during the dilution process.

- For Aqueous/Polar Systems:
  - Filtering or centrifuging some clear liquid from the original sample and using this to dilute the original concentrated sample.
  - Let the sample naturally sediment and use the fine particles left in the supernatant
  - Imitate the original medium as closely as possible with respect to pH, the total ionic concentration of the system, and concentration of any surfactant or polymers present.
- For Non-Polar Systems:
  - Universal dip cell is required because of its chemical compatibility and the close spacing of the electrodes allows the generation of high field strengths without using excessively high voltages.
  - Sample preparation is the same as polar systems. However, equilibration of the sample after dilution is time dependent step and can take more than 24 hrs.

<u>Cell Selection</u>

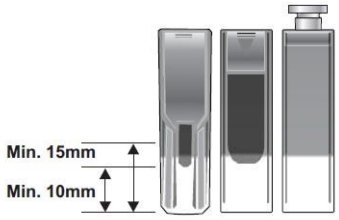
Optical quality glass or quartz cells should be used when performing molecular weight and protein measurements to ensure optimum signal is achieved. Also, **non-water based samples should generally be measured in glass or quartz type cuvettes**.

Disposable cells are commonly used for easy measurements; however, for best results, they should only be used once since they are easily scratched and are not resistant to organic solvents.

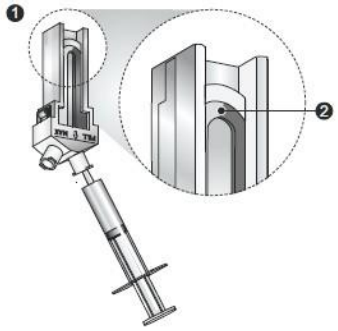
Cell #	Cell Type	Appropriate Solvents	Min. Volume	Temp. Range	Measurement Type
Sarstedt (67.754)	Disposable Polystyrene	Water, Water/ethanol	250 mL	< 50°C	Size
Brand Tech (759200)	Disposable Polystyrene	Water, Water/ethanol	70 mL	< 50°C	Size
Malvern (ZEN0040)	Disposable Polystyrene	Water, Water/ethanol	40 mL	< 70°C	Size
Malvern (PCS1115)	Glass – square aperture	Water, most organic and inorganic solvents	1000 mL	< 90°C	Size, Molecular Weight
Malvern (DTS1070)	Polycarbonate Folded Capillary Cell	Water, Water/ethanol	750 mL	< 70°C	Size, zeta potential

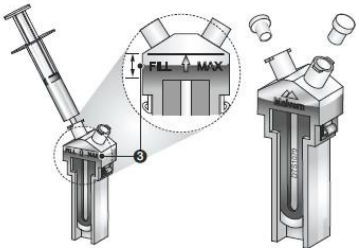
Filling the Cell

- Select a proper cell and rinse/clean with filtered dispersant. Molecular weight cells should be rinsed with filtered standard or solvent first.
- Fill the cell slowly to avoid the formation of air bubbles. If possible, use ultrasonication to remove air bubbles.
- If using a syringe filter, discard the first few drops in case there are residual dust particles from the filter.
- For size and molecular weight measurements fill the cell to have a sample height of between 10 to 15 mm from the bottom. Anything higher than 15 mm will produce thermal gradients reducing the temperature control accuracy. Readings are made at 8mm from the bottom of the cell.



- For zeta potential measurements using the folded capillary cell fill as follows:
  - Fill a syringe with 1mL of the sample
  - Insert the syringe into one of the ports
  - Invert the cell and slowly inject the sample until the U tube is half full
  - Check that there are no air bubbles in the cell. If so, tap until they are dislodged
  - Turn the cell upright and continue to inject slowly until the sample reaches the fill max line
  - Check again for air bubbles
  - Check that the electrodes are completely immersed
  - Remove syringe and insert stoppers in each port
  - Clean electrode contacts

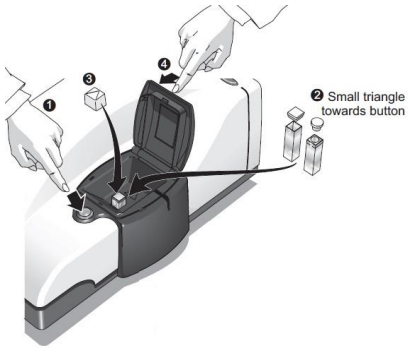




Inserting the Cell

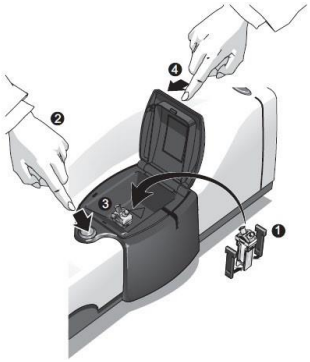
Regular Cells

1. Press silver button to open the latch to the cell area
2. Insert the regular cells into the cell holder with the triangle indicator on the cell facing towards you (or the open button). Push all the way down. The polished optical surface, indicated by the triangle, should face the front.
3. Place the thermal cap over the cell.
4. Close the cell area lid



Folded Capillary Cells

1. Place the thermal contact plate into the recess on either side of the folded capillary cell to provide temperature stability.
2. Press silver button to open the latch to the cell area
3. Insert the cell into the cell holder and push all the way down. It can be inserted either way.
4. Close cell area lid

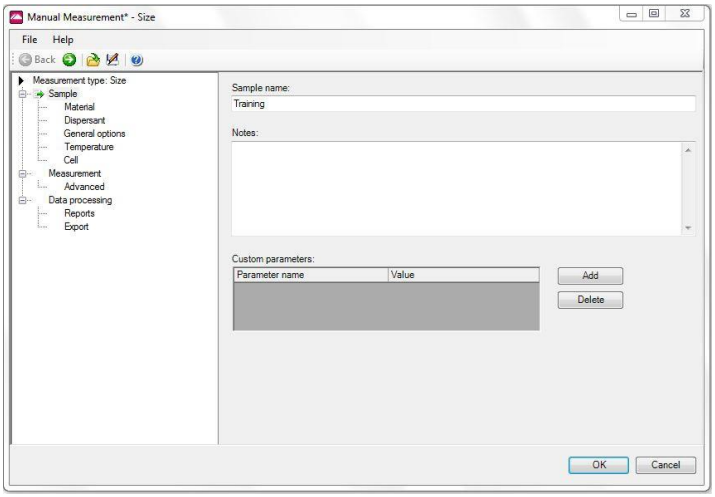


Manual Measurements

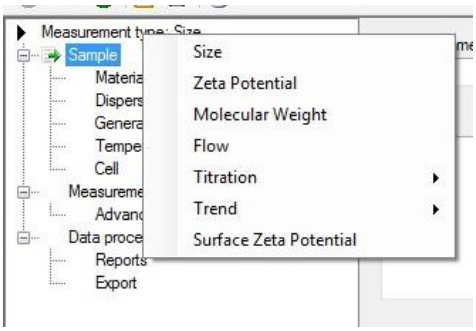
- Turn on the instrument using the switch at the back of the unit by the power cord. The instrument will beep three times when the system is ready.

- Start the Zetasizer Nano software using the icon 

on the desktop. On the Instrument, the light around the silver button to open the sample chamber lid will turn green. If it remains red please contact technician.
- Create a new measurement file through the **File->New->Measurement** menu. Provide a name for the measurement file in the dialog box, specify where to save the data and press Save.
- In the top menu select **Measure->Manual** to open the manual measurement setting dialog box.



- Follow the tabs and change any properties as needed. If unsure about anything, use the default setting(s):



a) Measurement type:
   - Size, zeta potential, or molecular weight.

b) Sample
   - Provide a sample name. This will label your data as well.

c) Material
   - Select the material you are measuring from the list.
   - If the material has not been listed, you will need to find the refractive index and absorption for that material.

d) Dispersant
   - Select the liquid your sample is dispersed in from the list.
   - If the dispersant you are using has not been listed, you will need to find the viscosity, refractive index, and (for zeta potential measurements) dielectric constant for that material.

e) General Options
   - Choices depend on measurement type. Here you will select the model to use when calculating the parameter (size, MW, zeta potential etc.)

f) Temperature
   - Set the desired temperature to run the sample. Note the temperature limits for each cuvette listed on page 2 of these instructions.
   - If the temperature is being changed from 25°C, or if your sample has recently been stored at a different temperature, give the sample 3-5 minutes to equilibrate.

g) Cell
   - Select the correct cell that you are using. Look at the instructions provided on selecting the correct cell type.

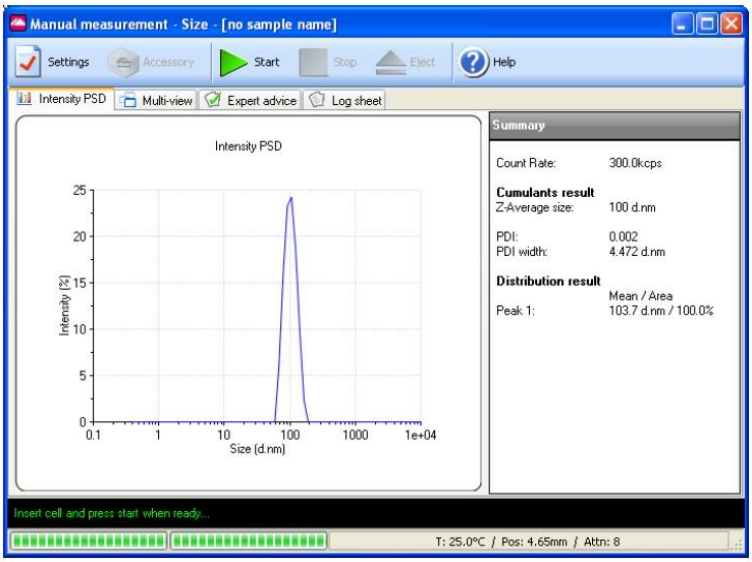
h) Measurement
   - Set the number of runs per measurement to Automatic unless your sample is changing over time (fewer runs) or if it is somewhat polydisperse (more runs)
   - More than one measurement is recommended to verify the consistency of your results.

i) Advanced
   - These settings should be at the default and do not need to be changed.

j) Data Processing
   - Choices depend on measurement type.

k) Report/Export

- Save as SOP if you want to save these settings for future experiments
- Click Ok
- Measurement window will now show on screen. Instructions and information will display in green text in the lower left-hand corner.
- Press Start to begin a measurement.
- To go back to the measurement parameters, click Settings.
- When the measurement is complete the system will beep once. New measurement record will be added to the Records view of the measurement file window. Highlight all the records from one measurement and right-click and choose Create Average Result to generate once line of average data. Select the average result line to view the reports of the averaged data.



- Save the file again to retain the average results.
- Save or print the report. Transfer all data to your server account.

Cleanup

- Remove the cuvette and recover or dispose of the sample appropriately.
- Close the software
- Clean the cuvette. Do not use abrasive material that could scratch the surfaces.
   - For all cuvettes, use the same solvent that was used in the measurement for washing
   - Zeta potential cuvettes can be cleaned by using a Luer-lock syringe and washing 3 times using the same solvent as the experiment, followed by 3 washing of highly purified water. Push air using the syringe to dry out the capillary.