

Standard preparation Protocol for Zeta-potential #23A1010

1. Prepare 15 mL of fresh 5 mM NaOH solution on the day of use.
2. Filter through 0.2 μm pore size filter to remove contaminant particles. Avoid using cellulose type membranes for filtration.
3. Transfer 4 mL mM NaOH solution to a new tube and add 2 μL of Zeta-potential standard stock (1:2000 dilution). Gently mix for 3 seconds using vortex on low setting.
4. Solution is stable for ~ 1 h at room temperature, some events of aggregation may occur with extended incubation.
5. Before starting a measurement:
 - Replace one of the supply bottle content with freshly filtered through 0.2 μm pore size filter DI water
 - Start the instrument and prime the cell using corresponding pump to fill the cell with fresh particle free DI water
 - Perform autoalignment with 100nm beads standard. Autoalignment must pass with message "microscope is ready to use". Plotted curve, shown after autolignment in "analysis" tab should be U-shaped and having close start and end points on left and right.
 - Flush 100nm calibration beads using 3-mL syringe, filled 5 mM NaOH solution
 - Less than 10 particles should be on the screen after flushing with 5 mM NaOH solution
 - Increase sensitivity to 80, shutter at 100; turn on "digital mode" to see in red what instrument interpret as particles. Switch position to 0.1 and then to 0.9, while microscope is moving, observe there is no background noise or bubbles. If noted any – repeat priming with pump or clean the cell before making ZP-measurement.
 - Set temperature to 25 C and incubate for 10 min.
7. Using 3 mL syringe filled with prepared ZP-standard, inject $\sim 1,5$ mL of solution into the cell.
8. Check "particles drift" is within expected range, incubate sample in the cell if necessary until equilibration.
9. Click on "update conductivity", result should be under 500 μS , otherwise NaOH concentration is incorrect, water supply has high salt content or air bubble is injected into the cell. In that case, ZP can be measured incorrectly.
10. Check your Zetaview SOP settings, correct if necessary:

Parameter	Value	Parameter	Value
Experiment	Zetapot.	ZP/Class	2.0
Mode	Continuous	Max ZP	200.0
Positions	11	Sensitivity	60
Min Brightness	30	Frame rate	30
Max Area	1000	Shutter	150
Min Area	10		
Tracelength	15		

11. Perform measurement, expected results range is – 50 mV \pm 10 mV: 10% deviation is acceptable error for standard and 10 % deviation is acceptable error for instrument.
12. Store ZP standard at +4 C.