

## Avoiding Artifacts in EV samples: Part 1 – the Use of Tween®

Many different methods have been developed for preparation of EVs, before characterization. Along with generally accepted methods are some “tricks of the trade” such as the addition of Tween® or BSA; however, some of those additions are problematic, in that they create significant background particles.

The use of Tween® in low concentrations for EV sample preparation has been recommended by several vendors. The creation of background particles may come as a surprise to some researchers, where filtration before addition to a sample may not entirely solve the issue for a couple of reasons. First, many syringe filters shed particles of their own and require conditioning before filtration of any diluent; therefore, one must be careful not to compound the problem when taking this step. Second, as Tween® is essentially detergent, it is likely that the interaction of Tween® with the protein in EV samples will generate additional, measurable particles after a filtration step. We present here some simple tests to illustrate the problem.

Figure 1 shows NTA measurement of just PBS, a null set. Only three particles were tracked when using a typical SOP for EVs, so this PBS is clean. Figures 2, 3 and 4 show the effect of adding Tween® to PBS, even though the additive is only present in low amounts (0.1% and 0.3% concentrations). Notably, our ZetaView® system is too sensitive at a concentration of 0.5% Tween®, which creates so many surfactant nanoparticles that NTA analysis isn't feasible.

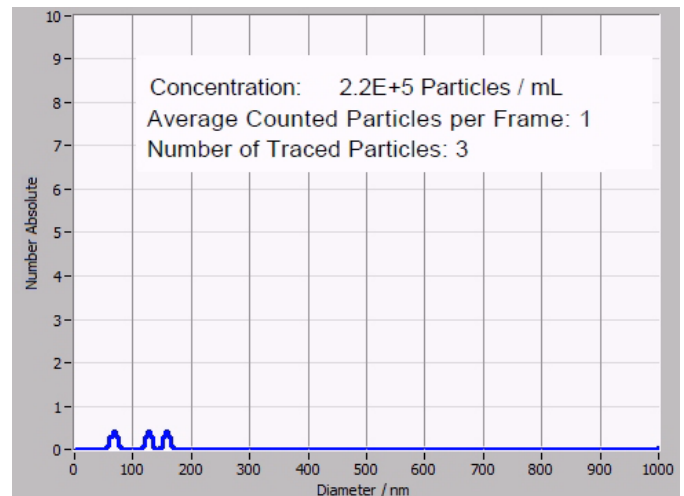


Figure 1: Analysis of PBS buffer without EVs, using ZetaView® 488 system.

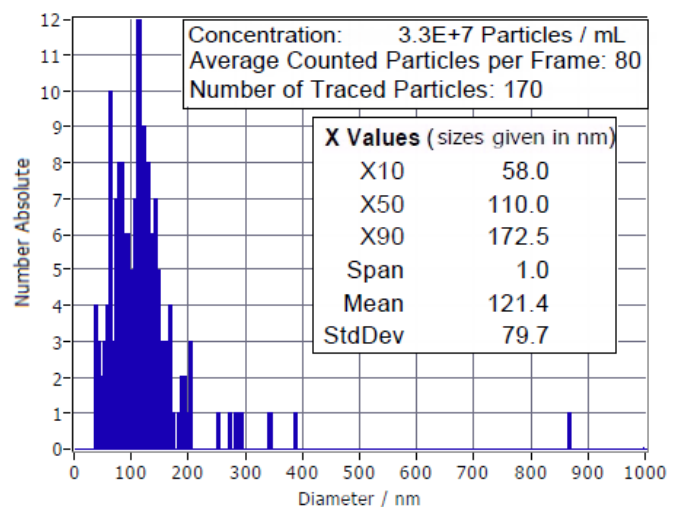


Figure 2: Analysis of PBS buffer containing 0.1% Tween®, using ZetaView® 488 system. A significant number of particles were measured, without EVs.



Figure 3: *Partial frame from NTA movie of PBS buffer with 0.1% Tween<sup>®</sup>, this view is typical of particle counts seen for the corresponding PSD.*

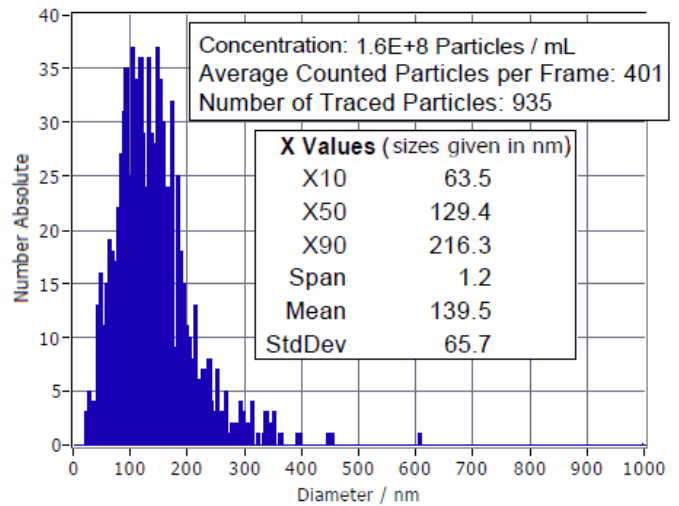


Figure 4: *Analysis of PBS buffer containing 0.3% Tween<sup>®</sup>, using ZetaView<sup>®</sup> 488 system. Enough particles are present in this buffer, even without EVs, such that this result closely resembles an NTA analysis of EVs!*

## Summary Advice

1. Don't use Tween<sup>®</sup> at all for EV prep. Why is that?  
While plenty of anecdotal evidence indicates that the the addition of Tween<sup>®</sup> will deteriorate the membrane of EVs, we see here that the sample chamber is being flooding with surfactant particles, which obscures measurement of EVs.
2. In any kind of experiments comparison, please take steps for blank control to ensure that methods in use are not actually generating additional particles which are not EVs.

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