

Supplementary information

CM-Dil Staining and SEC of Plasma as an Approach to Increase Sensitivity of Extracellular Nanovesicles Quantification by Bead-Assisted Flow Cytometry

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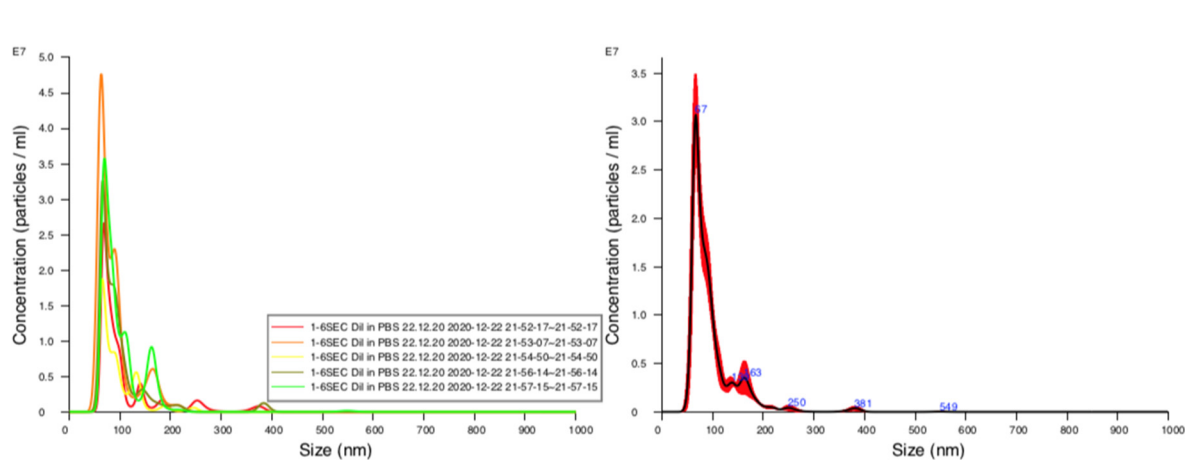
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Figure S1: CM-Dil, SEC fraction: 5-6

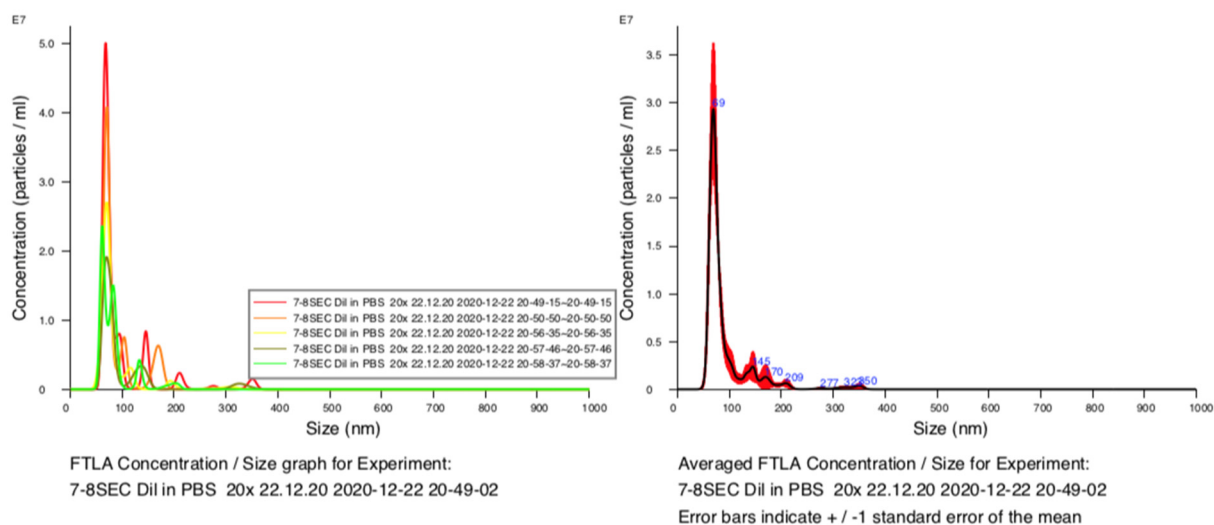


FTLA Concentration / Size graph for Experiment:
1-6SEC Dil in PBS 22.12.20 2020-12-22 21-51-35

Averaged FTLA Concentration / Size for Experiment:
1-6SEC Dil in PBS 22.12.20 2020-12-22 21-51-35
Error bars indicate + / -1 standard error of the mean

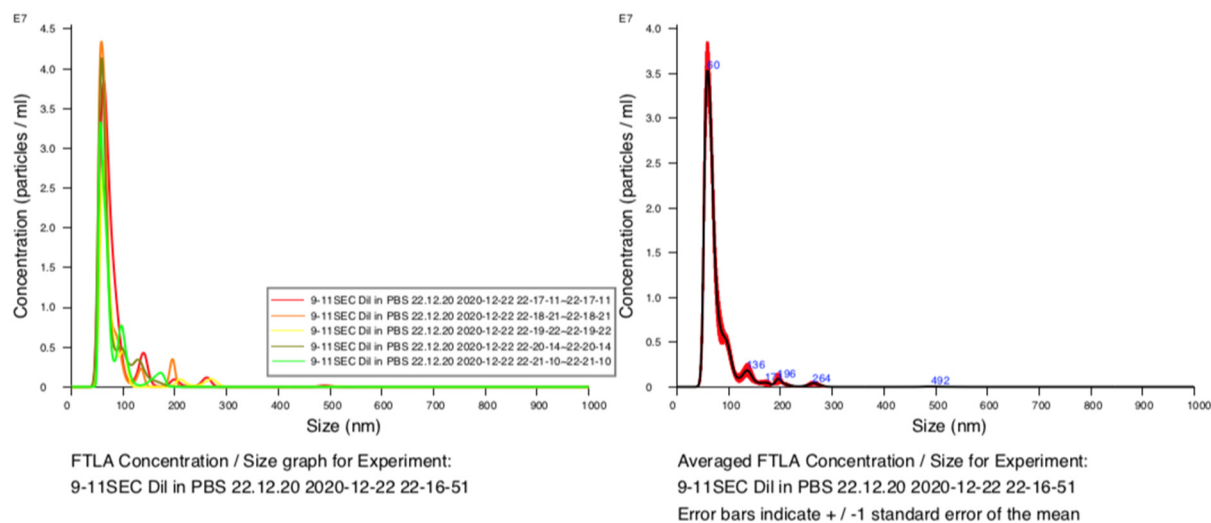
<p>Included Files</p> <p>1-6SEC Dil in PBS 22.12.20 2020-12-22 21-52-17 1-6SEC Dil in PBS 22.12.20 2020-12-22 21-53-07 1-6SEC Dil in PBS 22.12.20 2020-12-22 21-54-50 1-6SEC Dil in PBS 22.12.20 2020-12-22 21-56-14 1-6SEC Dil in PBS 22.12.20 2020-12-22 21-57-15</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 09-51-35PM 22~ Time Captured: 21:51:35 22/12/2020 Operator: Nadya Pre-treatment: Sample Name: 1-6SEC Dil in PBS 22.12.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 15 Slider Shutter: 1206 Slider Gain: 245 FPS: 25.0 Number of Frames: 749 Temperature: 25.3 - 25.5 °C Viscosity: (Water) 0.878 - 0.883 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 7 Blur Size: Auto Max Jump Distance: Auto: 15.7 - 17.2 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 96.7 nm Mode: 66.6 nm SD: 49.2 nm D10: 61.0 nm D50: 81.2 nm D90: 158.2 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 97.6 +/- 3.2 nm Mode: 66.4 +/- 1.2 nm SD: 48.7 +/- 5.7 nm D10: 61.8 +/- 1.5 nm D50: 81.3 +/- 1.5 nm D90: 159.2 +/- 8.5 nm</p> <p>Concentration (Upgrade): 1.25e+09 +/- 1.95e+08 particles/ml 73.9 +/- 10.1 particles/frame 76.5 +/- 8.5 centres/frame</p>
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Figure S2: CM-Dil, SEC fraction: 7-8



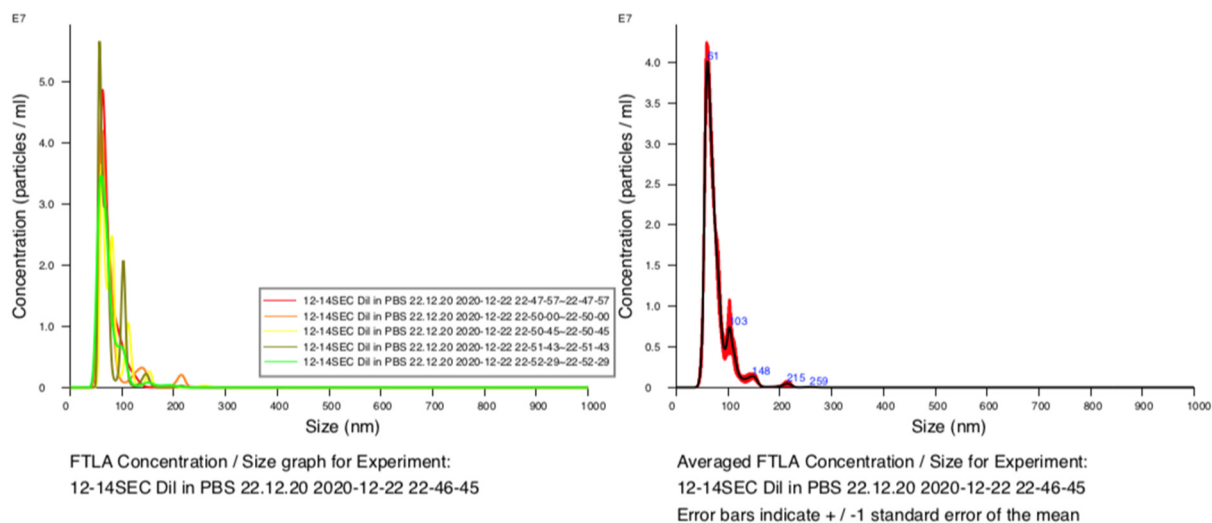
<p>Included Files</p> <p>7-8SEC Dil in PBS 20x 22.12.20 2020-12-22 20-49-15 7-8SEC Dil in PBS 20x 22.12.20 2020-12-22 20-50-50 7-8SEC Dil in PBS 20x 22.12.20 2020-12-22 20-56-35 7-8SEC Dil in PBS 20x 22.12.20 2020-12-22 20-57-46 7-8SEC Dil in PBS 20x 22.12.20 2020-12-22 20-58-37</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 08-49-02PM 22~ Time Captured: 20:49:02 22/12/2020 Operator: Nadya Pre-treatment: Sample Name: 7-8SEC Dil in PBS 20x 22.12.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 15 Slider Shutter: 1206 Slider Gain: 245 FPS: 25.0 Number of Frames: 749 Temperature: 24.9 - 25.3 °C Viscosity: (Water) 0.883 - 0.890 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 16.2 - 18.1 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 91.2 nm Mode: 68.9 nm SD: 49.1 nm D10: 61.4 nm D50: 73.6 nm D90: 147.6 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 90.8 +/- 2.8 nm Mode: 67.9 +/- 1.7 nm SD: 47.0 +/- 5.6 nm D10: 61.4 +/- 0.9 nm D50: 73.9 +/- 0.7 nm D90: 141.9 +/- 8.0 nm</p> <p>Concentration (Upgrade): 7.95e+08 +/- 1.02e+08 particles/ml 27.2 +/- 3.5 particles/frame 28.6 +/- 3.6 centres/frame</p>
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Figure S3: CM-Dil, SEC fraction: 9-11



<p>Included Files</p> <p>9-11SEC Dil in PBS 22.12.20 2020-12-22 22-17-11 9-11SEC Dil in PBS 22.12.20 2020-12-22 22-18-21 9-11SEC Dil in PBS 22.12.20 2020-12-22 22-19-22 9-11SEC Dil in PBS 22.12.20 2020-12-22 22-20-14 9-11SEC Dil in PBS 22.12.20 2020-12-22 22-21-10</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 10-16-51PM 22~ Time Captured: 22:16:51 22/12/2020 Operator: Nadya Pre-treatment: Sample Name: 9-11SEC Dil in PBS 22.12.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 15 Slider Shutter: 1206 Slider Gain: 245 FPS: 25.0 Number of Frames: 749 Temperature: 25.7 - 25.9 °C Viscosity: (Water) 0.871 - 0.874 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 7 Blur Size: Auto Max Jump Distance: Auto: 17.2 - 18.5 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 78.2 nm Mode: 59.2 nm SD: 40.5 nm D10: 53.7 nm D50: 65.6 nm D90: 112.4 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 78.3 +/- 2.3 nm Mode: 59.3 +/- 1.2 nm SD: 38.9 +/- 6.4 nm D10: 53.9 +/- 0.5 nm D50: 65.6 +/- 1.0 nm D90: 112.6 +/- 6.0 nm</p> <p>Concentration (Upgrade): 9.78e+08 +/- 9.32e+07 particles/ml 52.3 +/- 5.3 particles/frame 52.7 +/- 4.7 centres/frame</p>
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Figure S4: CM-Dil, SEC fraction: 12-13



Included Files

12-14SEC Dil in PBS 22.12.20 2020-12-22 22-47-57
12-14SEC Dil in PBS 22.12.20 2020-12-22 22-50-00
12-14SEC Dil in PBS 22.12.20 2020-12-22 22-50-45
12-14SEC Dil in PBS 22.12.20 2020-12-22 22-51-43
12-14SEC Dil in PBS 22.12.20 2020-12-22 22-52-29

Details

NTA Version: NTA 3.4 Build 3.4.003
Script Used: SOP Standard Measurement 10-46-45PM 22~
Time Captured: 22:46:45 22/12/2020
Operator: Nadya
Pre-treatment:
Sample Name: 12-14SEC Dil in PBS 22.12.20
Diluent: PBS
Remarks:

Capture Settings

Camera Type: sCMOS
Laser Type: Blue488
Camera Level: 15
Slider Shutter: 1206
Slider Gain: 245
FPS: 25.0
Number of Frames: 749
Temperature: 26.1 - 26.2 °C
Viscosity: (Water) 0.866 - 0.868 cP
Dilution factor: Dilution not recorded

Analysis Settings

Detect Threshold: 7
Blur Size: Auto
Max Jump Distance: Auto: 17.7 - 18.3 pix

Results

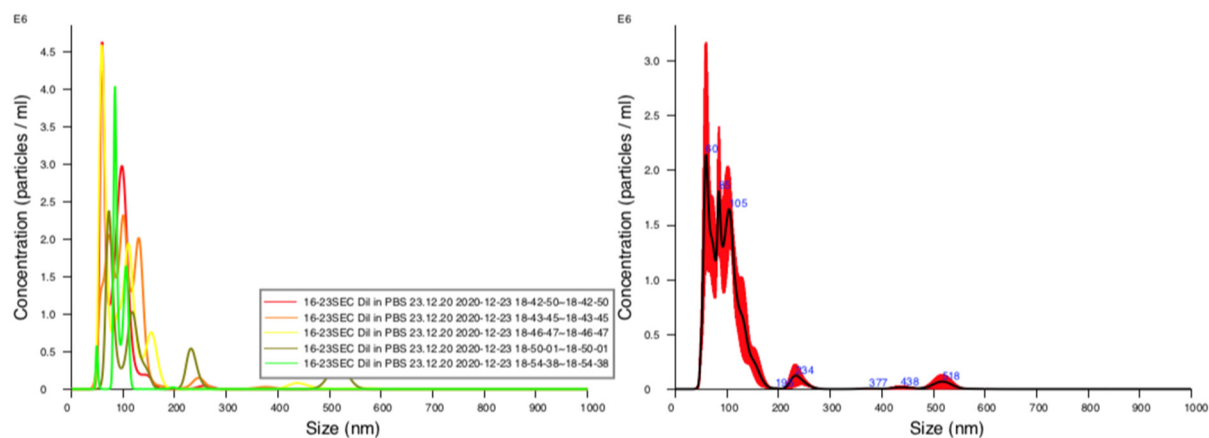
Stats: Merged Data

Mean: 76.0 nm
Mode: 60.2 nm
SD: 26.3 nm
D10: 55.4 nm
D50: 67.9 nm
D90: 106.5 nm

Stats: Mean +/- Standard Error

Mean: 76.2 +/- 1.7 nm
Mode: 60.3 +/- 1.0 nm
SD: 25.7 +/- 2.5 nm
D10: 55.6 +/- 1.0 nm
D50: 68.6 +/- 1.7 nm
D90: 109.2 +/- 5.3 nm
Concentration (Upgrade): 1.13e+09 +/- 4.53e+07 particles/ml
50.0 +/- 1.7 particles/frame
51.0 +/- 1.8 centres/frame

Figure S5: CM-Dil, SEC fraction: 16-21

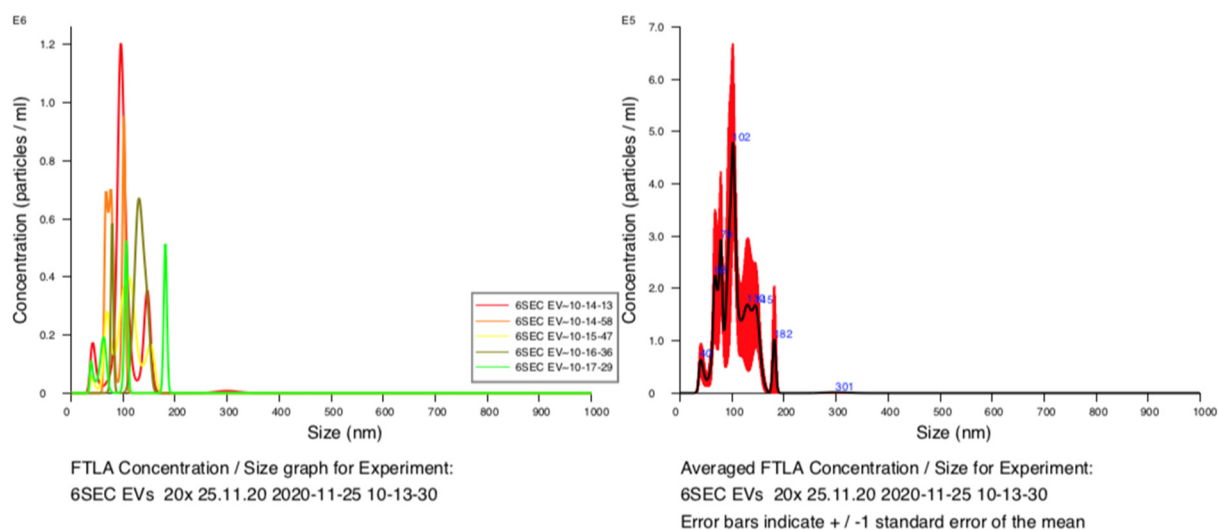


FTLA Concentration / Size graph for Experiment:
16-23SEC Dil in PBS 23.12.20 2020-12-23 18-41-47

Averaged FTLA Concentration / Size for Experiment:
16-23SEC Dil in PBS 23.12.20 2020-12-23 18-41-47
Error bars indicate + / - 1 standard error of the mean

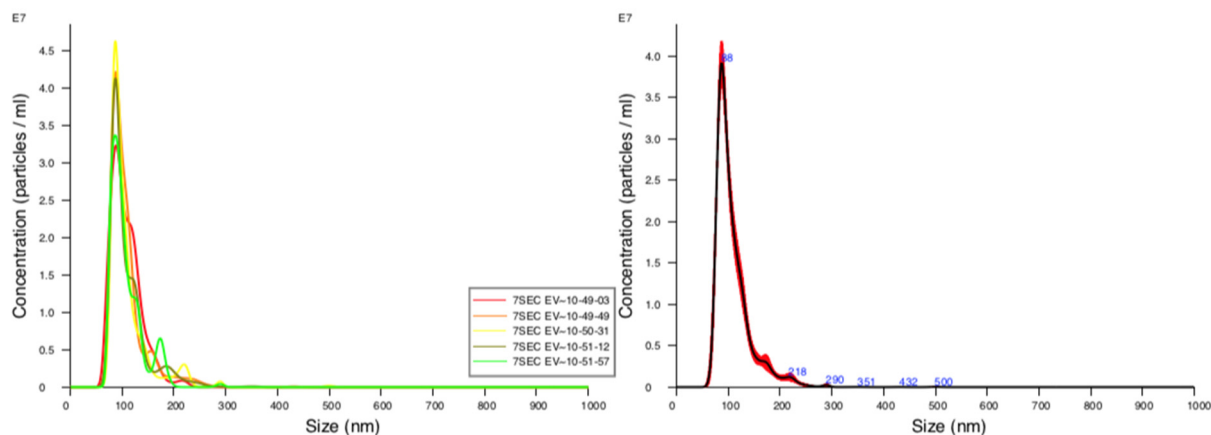
<p>Included Files</p> <p>16-23SEC Dil in PBS 23.12.20 2020-12-23 18-42-50 16-23SEC Dil in PBS 23.12.20 2020-12-23 18-43-45 16-23SEC Dil in PBS 23.12.20 2020-12-23 18-46-47 16-23SEC Dil in PBS 23.12.20 2020-12-23 18-50-01 16-23SEC Dil in PBS 23.12.20 2020-12-23 18-54-38</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 06-41-47PM 23~ Time Captured: 18:41:47 23/12/2020 Operator: Nadya Pre-treatment: Sample Name: 16-23SEC Dil in PBS 23.12.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 16 Slider Shutter: 1300 Slider Gain: 295 FPS: 25.0 Number of Frames: 749 Temperature: 24.5 - 25.0 °C Viscosity: (Water) 0.888 - 0.900 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 7 Blur Size: Auto Max Jump Distance: Auto: 14.2 - 19.4 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 110.4 nm Mode: 59.3 nm SD: 80.9 nm D10: 58.7 nm D50: 93.1 nm D90: 148.3 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 112.7 +/- 16.3 nm Mode: 75.1 +/- 7.9 nm SD: 62.6 +/- 24.0 nm D10: 65.4 +/- 4.6 nm D50: 95.7 +/- 5.2 nm D90: 204.5 +/- 76.5 nm</p> <p>Concentration (Upgrade): 1.30e+08 +/- 2.18e+07 particles/ml 9.2 +/- 1.5 particles/frame 9.6 +/- 1.5 centres/frame</p>
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Figure S6: PL, SEC fraction: 5-6



<p>Included Files</p> <p>6SEC EVs 20x 25.11.20 2020-11-25 10-14-13 6SEC EVs 20x 25.11.20 2020-11-25 10-14-58 6SEC EVs 20x 25.11.20 2020-11-25 10-15-47 6SEC EVs 20x 25.11.20 2020-11-25 10-16-36 6SEC EVs 20x 25.11.20 2020-11-25 10-17-29</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 10-13-30AM 25~ Time Captured: 10:13:30 25/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 6SEC EVs 20x 25.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 16 Slider Shutter: 1300 Slider Gain: 295 FPS: 25.0 Number of Frames: 749 Temperature: 22.9 °C Viscosity: (Water) 0.931 - 0.933 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 5 Blur Size: Auto Max Jump Distance: Auto: 15.1 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 105.8 nm Mode: 101.8 nm SD: 33.4 nm D10: 67.4 nm D50: 102.0 nm D90: 147.4 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 106.4 +/- 6.8 nm Mode: 108.9 +/- 5.9 nm SD: 30.2 +/- 6.2 nm D10: 69.1 +/- 4.9 nm D50: 103.8 +/- 8.3 nm D90: 145.3 +/- 12.6 nm</p> <p>Concentration (Upgrade): 2.18e+07 +/- 3.43e+06 particles/ml 1.7 +/- 0.2 particles/frame 2.1 +/- 0.3 centres/frame</p> <p>Concentration measurements may be unreliable See summary file for more info</p>
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Figure S7: PL, SEC fraction: 7

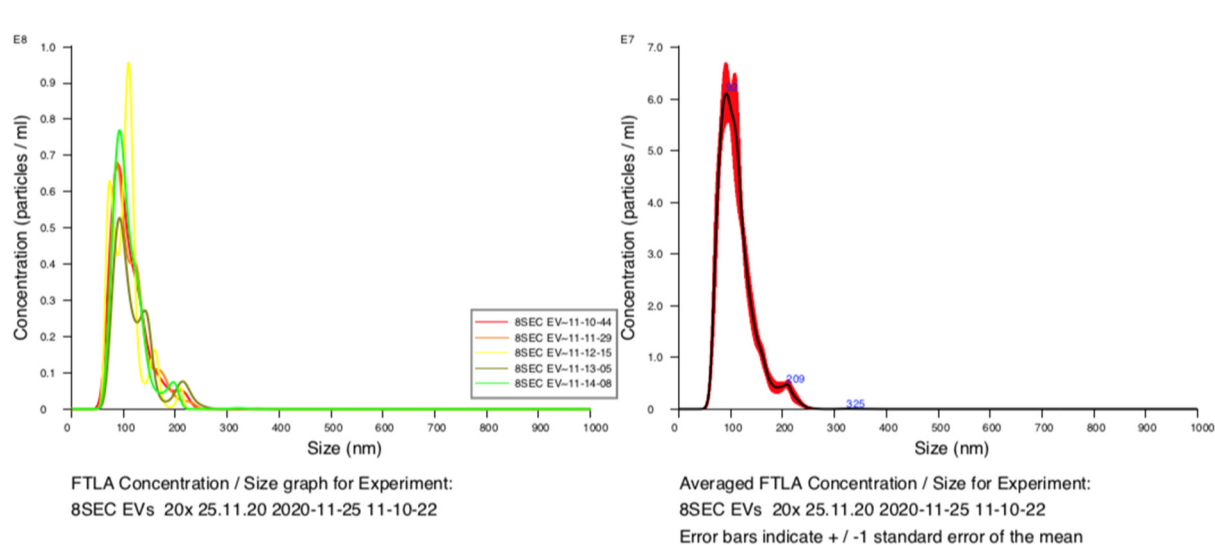


FTLA Concentration / Size graph for Experiment:
7SEC EVs 20x 25.11.20 2020-11-25 10-48-28

Averaged FTLA Concentration / Size for Experiment:
7SEC EVs 20x 25.11.20 2020-11-25 10-48-28
Error bars indicate + / - 1 standard error of the mean

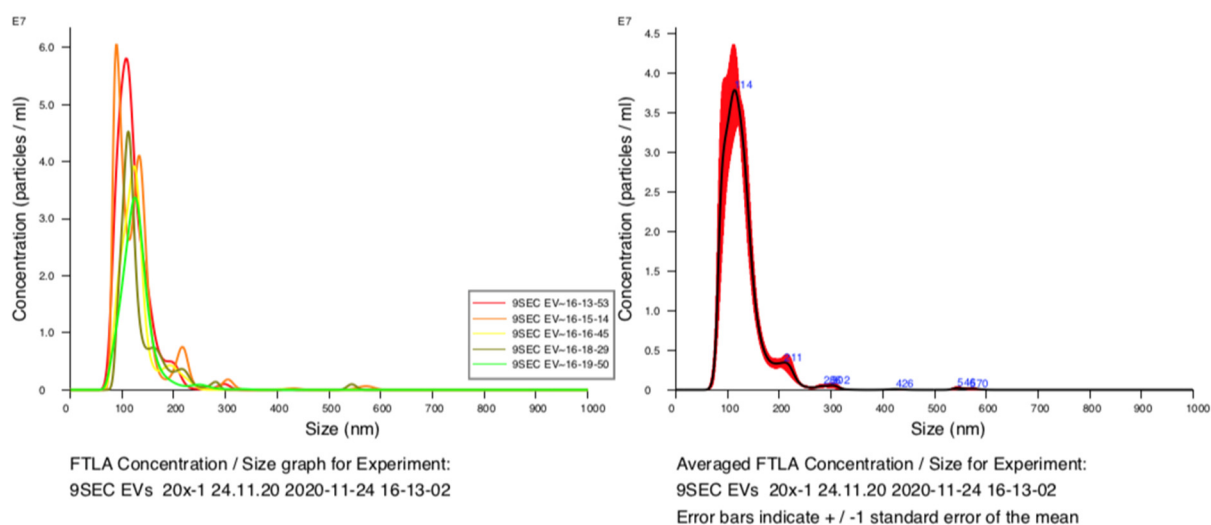
<p>Included Files</p> <p>7SEC EVs 20x 25.11.20 2020-11-25 10-49-03 7SEC EVs 20x 25.11.20 2020-11-25 10-49-49 7SEC EVs 20x 25.11.20 2020-11-25 10-50-31 7SEC EVs 20x 25.11.20 2020-11-25 10-51-12 7SEC EVs 20x 25.11.20 2020-11-25 10-51-57</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 10-48-28AM 25~ Time Captured: 10:48:28 25/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 7SEC EVs 20x 25.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 13 Slider Shutter: 1232 Slider Gain: 175 FPS: 25.0 Number of Frames: 749 Temperature: 24.3 - 24.5 °C Viscosity: (Water) 0.899 - 0.902 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 14.8 - 15.1 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 108.8 nm Mode: 87.2 nm SD: 35.7 nm D10: 78.4 nm D50: 98.5 nm D90: 151.7 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 108.8 +/- 0.6 nm Mode: 87.3 +/- 0.3 nm SD: 35.4 +/- 2.3 nm D10: 78.4 +/- 0.7 nm D50: 98.6 +/- 0.9 nm D90: 153.5 +/- 2.3 nm</p> <p>Concentration (Upgrade): 1.75e+09 +/- 5.80e+07 particles/ml 61.9 +/- 2.5 particles/frame 63.0 +/- 2.5 centres/frame</p>
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Figure S8: PL, SEC fraction: 8



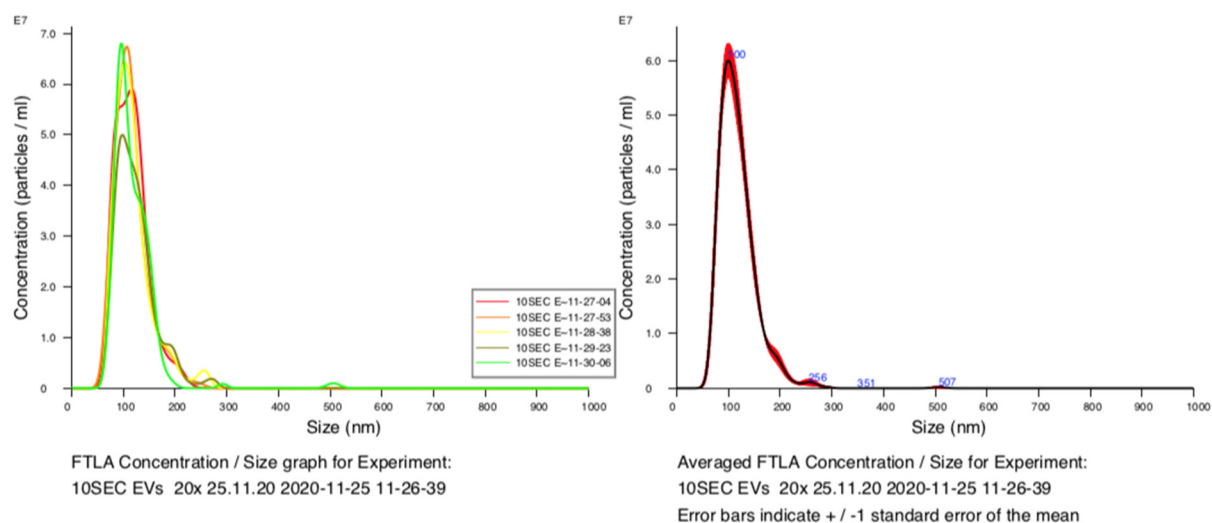
<p>Included Files</p> <p>8SEC EVs 20x 25.11.20 2020-11-25 11-10-44 8SEC EVs 20x 25.11.20 2020-11-25 11-11-29 8SEC EVs 20x 25.11.20 2020-11-25 11-12-15 8SEC EVs 20x 25.11.20 2020-11-25 11-13-05 8SEC EVs 20x 25.11.20 2020-11-25 11-14-08</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 11-10-22AM 25~ Time Captured: 11:10:22 25/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 8SEC EVs 20x 25.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 13 Slider Shutter: 1232 Slider Gain: 175 FPS: 25.0 Number of Frames: 749 Temperature: 24.4 - 24.4 °C Viscosity: (Water) 0.900 - 0.901 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 13.9 - 14.9 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 111.2 nm Mode: 91.3 nm SD: 34.4 nm D10: 75.8 nm D50: 104.0 nm D90: 156.5 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 111.6 +/- 2.4 nm Mode: 94.2 +/- 4.0 nm SD: 34.2 +/- 1.9 nm D10: 76.4 +/- 1.6 nm D50: 104.4 +/- 1.1 nm D90: 157.4 +/- 5.1 nm</p> <p>Concentration (Upgrade): 4.03e+09 +/- 1.97e+08 particles/ml 190.3 +/- 6.3 particles/frame 166.7 +/- 4.0 centres/frame</p>
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Figure S9: PL, SEC fraction: 9



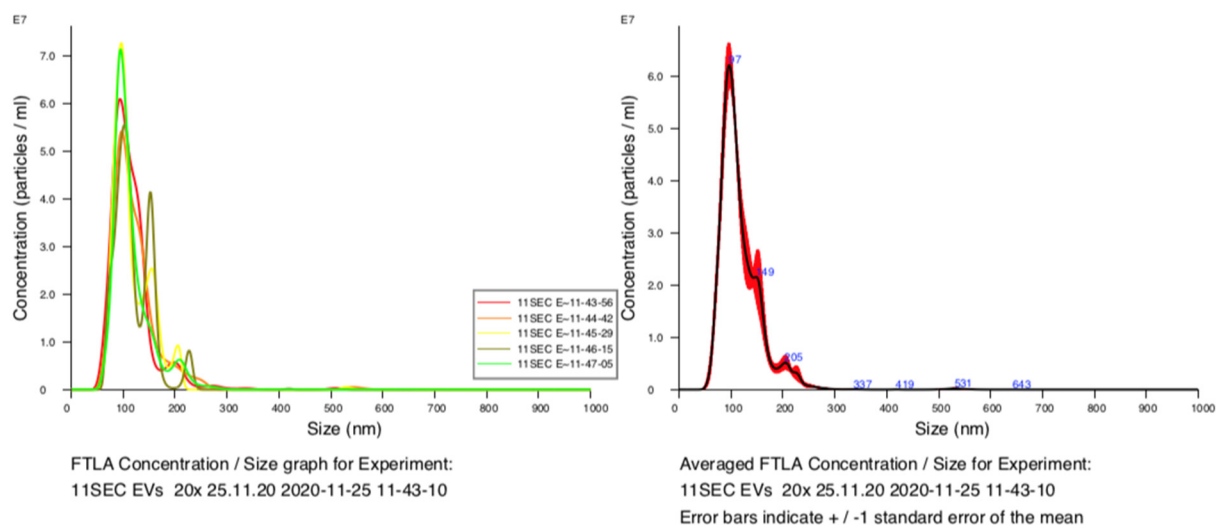
<p>Included Files</p> <p>9SEC EVs 20x-1 24.11.20 2020-11-24 16-13-53 9SEC EVs 20x-1 24.11.20 2020-11-24 16-15-14 9SEC EVs 20x-1 24.11.20 2020-11-24 16-16-45 9SEC EVs 20x-1 24.11.20 2020-11-24 16-18-29 9SEC EVs 20x-1 24.11.20 2020-11-24 16-19-50</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 04-13-02PM 24~ Time Captured: 16:13:02 24/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 9SEC EVs 20x-1 24.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 12 Slider Shutter: 1200 Slider Gain: 125 FPS: 25.0 Number of Frames: 1498 Temperature: 25.0 - 25.1 °C Viscosity: (Water) 0.886 - 0.889 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 13.7 - 14.2 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 127.9 nm Mode: 113.6 nm SD: 46.6 nm D10: 89.6 nm D50: 118.8 nm D90: 172.4 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 128.7 +/- 2.3 nm Mode: 111.7 +/- 6.4 nm SD: 44.3 +/- 6.0 nm D10: 91.9 +/- 2.1 nm D50: 119.3 +/- 1.9 nm D90: 176.2 +/- 6.6 nm</p> <p>Concentration (Upgrade): 2.53e+09 +/- 3.20e+08 particles/ml 89.6 +/- 14.7 particles/frame 89.7 +/- 14.1 centres/frame</p>
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Figure S10: PL, SEC fraction: 10



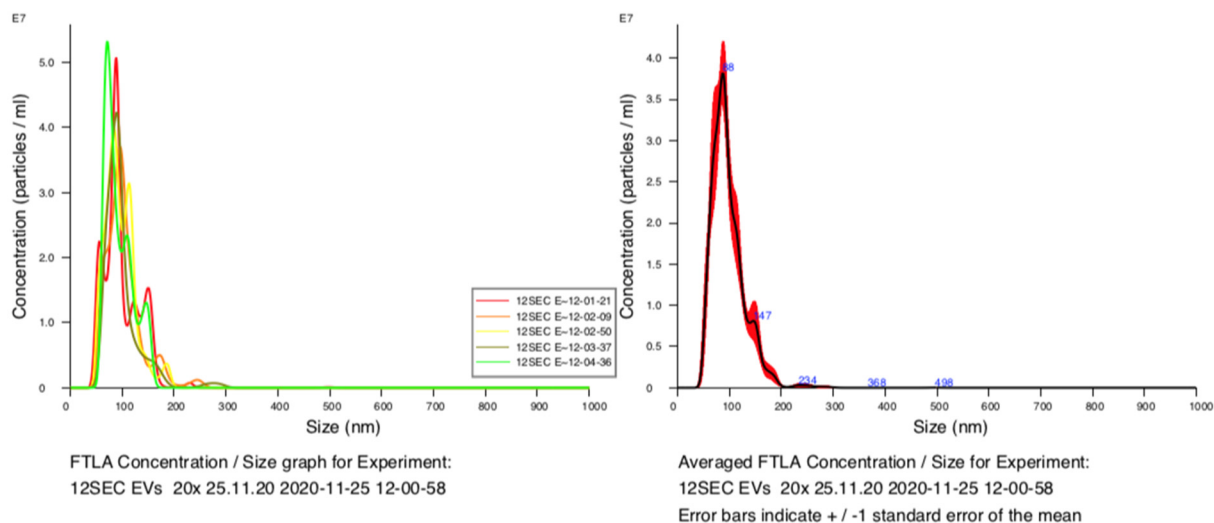
<p>Included Files</p> <p>10SEC EVs 20x 25.11.20 2020-11-25 11-27-04 10SEC EVs 20x 25.11.20 2020-11-25 11-27-53 10SEC EVs 20x 25.11.20 2020-11-25 11-28-38 10SEC EVs 20x 25.11.20 2020-11-25 11-29-23 10SEC EVs 20x 25.11.20 2020-11-25 11-30-06</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 11-26-39AM 25~ Time Captured: 11:26:39 25/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 10SEC EVs 20x 25.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 12 Slider Shutter: 1200 Slider Gain: 125 FPS: 25.0 Number of Frames: 749 Temperature: 24.3 - 24.3 °C Viscosity: (Water) 0.902 - 0.904 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 14.1 - 14.8 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <table> <tr><td>Mean:</td><td>117.9 nm</td></tr> <tr><td>Mode:</td><td>99.7 nm</td></tr> <tr><td>SD:</td><td>38.1 nm</td></tr> <tr><td>D10:</td><td>79.8 nm</td></tr> <tr><td>D50:</td><td>111.4 nm</td></tr> <tr><td>D90:</td><td>161.6 nm</td></tr> </table> <p>Stats: Mean +/- Standard Error</p> <table> <tr><td>Mean:</td><td>118.1 +/- 1.4 nm</td></tr> <tr><td>Mode:</td><td>103.9 +/- 3.5 nm</td></tr> <tr><td>SD:</td><td>37.9 +/- 2.3 nm</td></tr> <tr><td>D10:</td><td>79.9 +/- 0.9 nm</td></tr> <tr><td>D50:</td><td>111.6 +/- 1.2 nm</td></tr> <tr><td>D90:</td><td>163.2 +/- 3.7 nm</td></tr> </table> <p>Concentration (Upgrade): 4.37e+09 +/- 1.42e+08 particles/ml 177.9 +/- 4.9 particles/frame 173.1 +/- 3.9 centres/frame</p>	Mean:	117.9 nm	Mode:	99.7 nm	SD:	38.1 nm	D10:	79.8 nm	D50:	111.4 nm	D90:	161.6 nm	Mean:	118.1 +/- 1.4 nm	Mode:	103.9 +/- 3.5 nm	SD:	37.9 +/- 2.3 nm	D10:	79.9 +/- 0.9 nm	D50:	111.6 +/- 1.2 nm	D90:	163.2 +/- 3.7 nm
Mean:	117.9 nm																								
Mode:	99.7 nm																								
SD:	38.1 nm																								
D10:	79.8 nm																								
D50:	111.4 nm																								
D90:	161.6 nm																								
Mean:	118.1 +/- 1.4 nm																								
Mode:	103.9 +/- 3.5 nm																								
SD:	37.9 +/- 2.3 nm																								
D10:	79.9 +/- 0.9 nm																								
D50:	111.6 +/- 1.2 nm																								
D90:	163.2 +/- 3.7 nm																								

Figure S11: PL, SEC fraction: 11



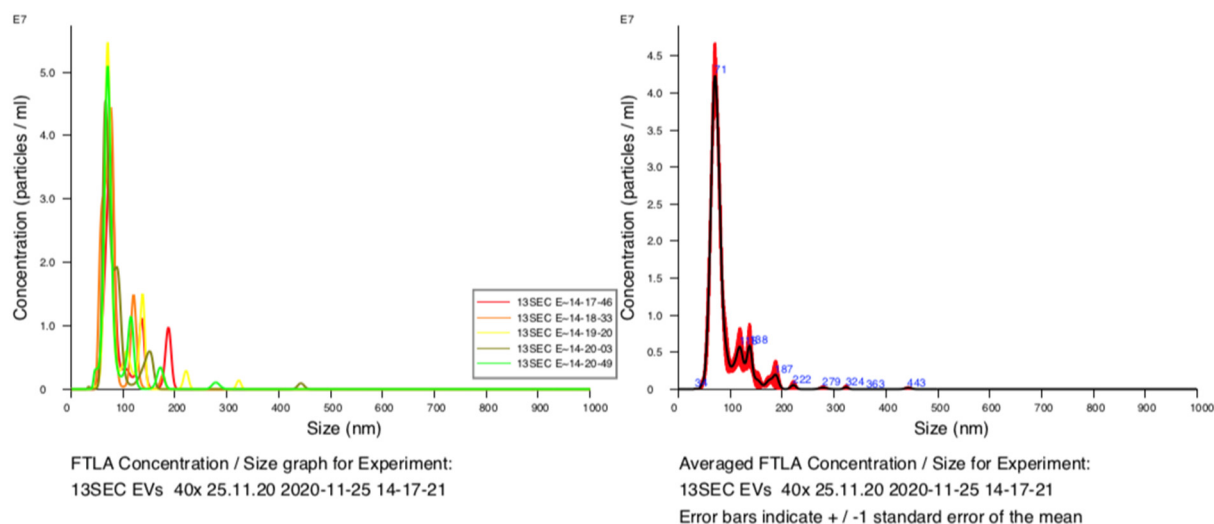
<p>Included Files</p> <p>11SEC EVs 20x 25.11.20 2020-11-25 11-43-56 11SEC EVs 20x 25.11.20 2020-11-25 11-44-42 11SEC EVs 20x 25.11.20 2020-11-25 11-45-29 11SEC EVs 20x 25.11.20 2020-11-25 11-46-15 11SEC EVs 20x 25.11.20 2020-11-25 11-47-05</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 11-43-10AM 25~ Time Captured: 11:43:10 25/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 11SEC EVs 20x 25.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 12 Slider Shutter: 1200 Slider Gain: 125 FPS: 25.0 Number of Frames: 749 Temperature: 24.3 - 24.4 °C Viscosity: (Water) 0.901 - 0.904 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 14.2 - 14.7 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 118.1 nm Mode: 96.6 nm SD: 43.4 nm D10: 79.6 nm D50: 107.4 nm D90: 164.3 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 118.1 +/- 1.3 nm Mode: 97.0 +/- 1.5 nm SD: 42.9 +/- 2.7 nm D10: 79.7 +/- 0.8 nm D50: 107.6 +/- 1.4 nm D90: 164.5 +/- 3.1 nm</p> <p>Concentration (Upgrade): 3.79e+09 +/- 1.04e+08 particles/ml 148.8 +/- 6.4 particles/frame 152.7 +/- 6.1 centres/frame</p>
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Figure S12: PL, SEC fraction: 12



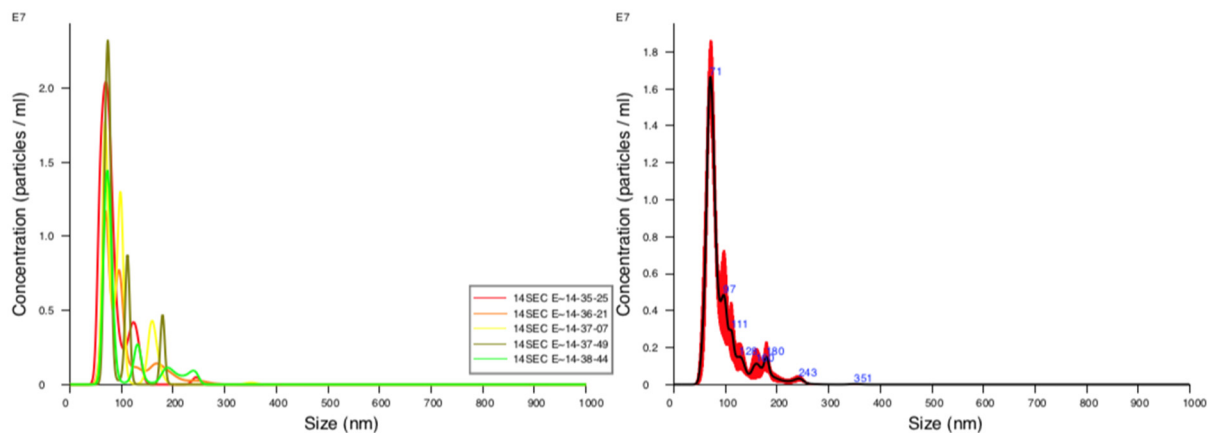
<p>Included Files</p> <p>12SEC EVs 20x 25.11.20 2020-11-25 12-01-21 12SEC EVs 20x 25.11.20 2020-11-25 12-02-09 12SEC EVs 20x 25.11.20 2020-11-25 12-02-50 12SEC EVs 20x 25.11.20 2020-11-25 12-03-37 12SEC EVs 20x 25.11.20 2020-11-25 12-04-36</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 12-00-58PM 25~ Time Captured: 12:00:58 25/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 12SEC EVs 20x 25.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 13 Slider Shutter: 1232 Slider Gain: 175 FPS: 25.0 Number of Frames: 749 Temperature: 24.2 - 24.3 °C Viscosity: (Water) 0.902 - 0.905 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 15.9 - 16.5 pix</p>	<p>Results</p> <p>Stats: Merged Data Mean: 97.7 nm Mode: 87.3 nm SD: 32.8 nm D10: 63.6 nm D50: 91.1 nm D90: 141.6 nm</p> <p>Stats: Mean +/- Standard Error Mean: 97.8 +/- 1.4 nm Mode: 85.7 +/- 4.0 nm SD: 32.6 +/- 1.9 nm D10: 63.4 +/- 1.7 nm D50: 90.9 +/- 1.6 nm D90: 141.0 +/- 3.4 nm Concentration (Upgrade): 2.23e+09 +/- 7.61e+07 particles/ml 66.5 +/- 2.6 particles/frame 83.2 +/- 3.1 centres/frame Concentration measurements may require some caution due to noise See summary file for more info</p>
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Figure S13: PL, SEC fraction: 13



<p>Included Files</p> <p>13SEC EVs 40x 25.11.20 2020-11-25 14-17-46 13SEC EVs 40x 25.11.20 2020-11-25 14-18-33 13SEC EVs 40x 25.11.20 2020-11-25 14-19-20 13SEC EVs 40x 25.11.20 2020-11-25 14-20-03 13SEC EVs 40x 25.11.20 2020-11-25 14-20-49</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 02-17-21PM 25~ Time Captured: 14:17:21 25/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 13SEC EVs 40x 25.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 14 Slider Shutter: 1259 Slider Gain: 245 FPS: 25.0 Number of Frames: 749 Temperature: 24.4 - 24.6 °C Viscosity: (Water) 0.897 - 0.900 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 16.2 - 17.4 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <table> <tr><td>Mean:</td><td>88.5 nm</td></tr> <tr><td>Mode:</td><td>70.6 nm</td></tr> <tr><td>SD:</td><td>38.9 nm</td></tr> <tr><td>D10:</td><td>61.7 nm</td></tr> <tr><td>D50:</td><td>75.1 nm</td></tr> <tr><td>D90:</td><td>135.7 nm</td></tr> </table> <p>Stats: Mean +/- Standard Error</p> <table> <tr><td>Mean:</td><td>88.7 +/- 3.0 nm</td></tr> <tr><td>Mode:</td><td>71.8 +/- 1.9 nm</td></tr> <tr><td>SD:</td><td>38.0 +/- 3.7 nm</td></tr> <tr><td>D10:</td><td>62.1 +/- 1.3 nm</td></tr> <tr><td>D50:</td><td>75.2 +/- 1.2 nm</td></tr> <tr><td>D90:</td><td>139.5 +/- 11.1 nm</td></tr> </table> <p>Concentration (Upgrade): 1.23e+09 +/- 3.86e+07 particles/ml 34.8 +/- 2.1 particles/frame 40.4 +/- 2.3 centres/frame</p>	Mean:	88.5 nm	Mode:	70.6 nm	SD:	38.9 nm	D10:	61.7 nm	D50:	75.1 nm	D90:	135.7 nm	Mean:	88.7 +/- 3.0 nm	Mode:	71.8 +/- 1.9 nm	SD:	38.0 +/- 3.7 nm	D10:	62.1 +/- 1.3 nm	D50:	75.2 +/- 1.2 nm	D90:	139.5 +/- 11.1 nm
Mean:	88.5 nm																								
Mode:	70.6 nm																								
SD:	38.9 nm																								
D10:	61.7 nm																								
D50:	75.1 nm																								
D90:	135.7 nm																								
Mean:	88.7 +/- 3.0 nm																								
Mode:	71.8 +/- 1.9 nm																								
SD:	38.0 +/- 3.7 nm																								
D10:	62.1 +/- 1.3 nm																								
D50:	75.2 +/- 1.2 nm																								
D90:	139.5 +/- 11.1 nm																								

Figure S14: PL, SEC fraction: 14

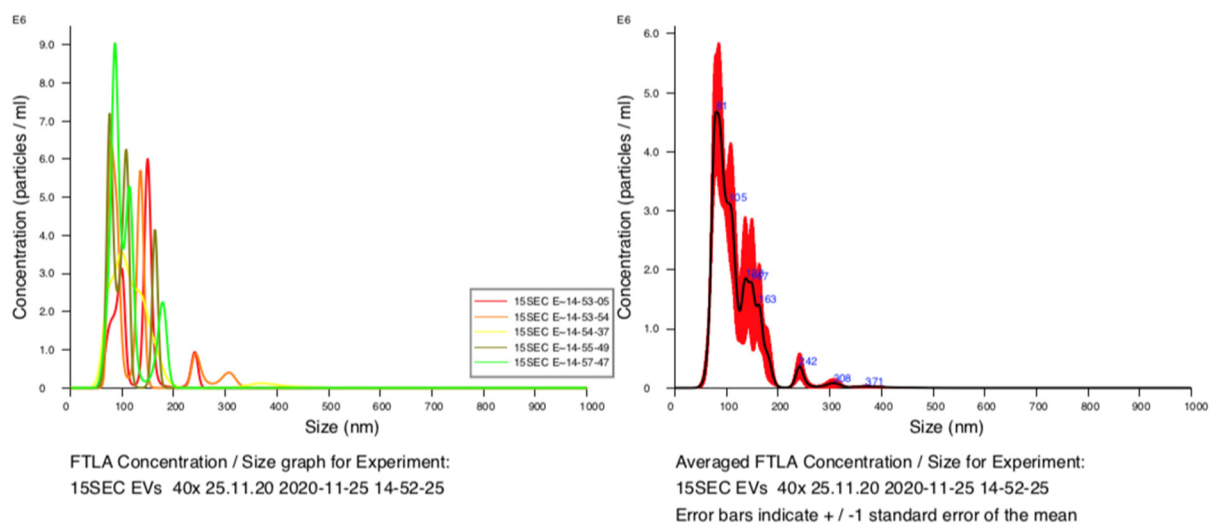


FTLA Concentration / Size graph for Experiment:
14SEC EVs 40x 25.11.20 2020-11-25 14-34-31

Averaged FTLA Concentration / Size for Experiment:
14SEC EVs 40x 25.11.20 2020-11-25 14-34-31
Error bars indicate + / -1 standard error of the mean

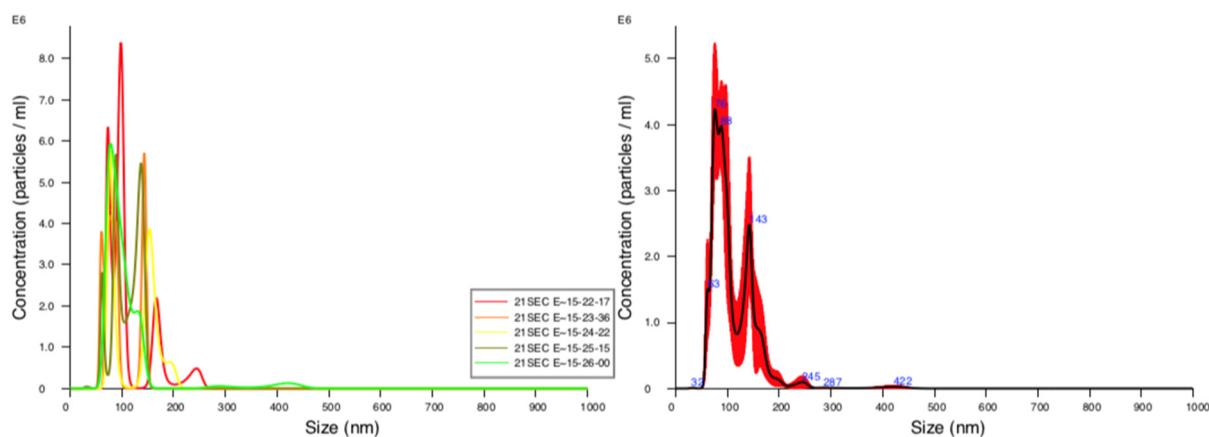
<p>Included Files</p> <p>14SEC EVs 40x 25.11.20 2020-11-25 14-35-25 14SEC EVs 40x 25.11.20 2020-11-25 14-36-21 14SEC EVs 40x 25.11.20 2020-11-25 14-37-07 14SEC EVs 40x 25.11.20 2020-11-25 14-37-49 14SEC EVs 40x 25.11.20 2020-11-25 14-38-44</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 02-34-31PM 25~ Time Captured: 14:34:31 25/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 14SEC EVs 40x 25.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 14 Slider Shutter: 1259 Slider Gain: 245 FPS: 25.0 Number of Frames: 749 Temperature: 24.8 °C Viscosity: (Water) 0.891 - 0.893 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 15.8 - 17.9 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 93.8 nm Mode: 70.7 nm SD: 39.9 nm D10: 63.0 nm D50: 78.3 nm D90: 156.0 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 95.3 +/- 4.1 nm Mode: 70.5 +/- 1.0 nm SD: 39.6 +/- 4.2 nm D10: 63.8 +/- 1.6 nm D50: 81.0 +/- 3.1 nm D90: 164.7 +/- 12.7 nm</p> <p>Concentration (Upgrade): 5.53e+08 +/- 6.03e+07 particles/ml 17.0 +/- 1.1 particles/frame 19.4 +/- 1.2 centres/frame</p>
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Figure S15: PL, SEC fraction: 15



<p>Included Files</p> <p>15SEC EVs 40x 25.11.20 2020-11-25 14-53-05 15SEC EVs 40x 25.11.20 2020-11-25 14-53-54 15SEC EVs 40x 25.11.20 2020-11-25 14-54-37 15SEC EVs 40x 25.11.20 2020-11-25 14-55-49 15SEC EVs 40x 25.11.20 2020-11-25 14-57-47</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 02-52-25PM 25~ Time Captured: 14:52:25 25/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 15SEC EVs 40x 25.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 13 Slider Shutter: 1232 Slider Gain: 175 FPS: 25.0 Number of Frames: 749 Temperature: 24.4 - 24.7 °C Viscosity: (Water) 0.895 - 0.901 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 14.4 - 16.1 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 116.7 nm Mode: 80.6 nm SD: 48.4 nm D10: 74.7 nm D50: 103.6 nm D90: 166.0 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 117.8 +/- 4.8 nm Mode: 98.0 +/- 13.5 nm SD: 46.1 +/- 6.4 nm D10: 75.2 +/- 1.1 nm D50: 109.4 +/- 8.4 nm D90: 180.5 +/- 15.1 nm Concentration (Upgrade): 2.90e+08 +/- 2.31e+07 particles/ml 9.7 +/- 0.4 particles/frame 10.6 +/- 0.4 centres/frame</p>
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Figure S16: PL, SEC fraction: 16-21

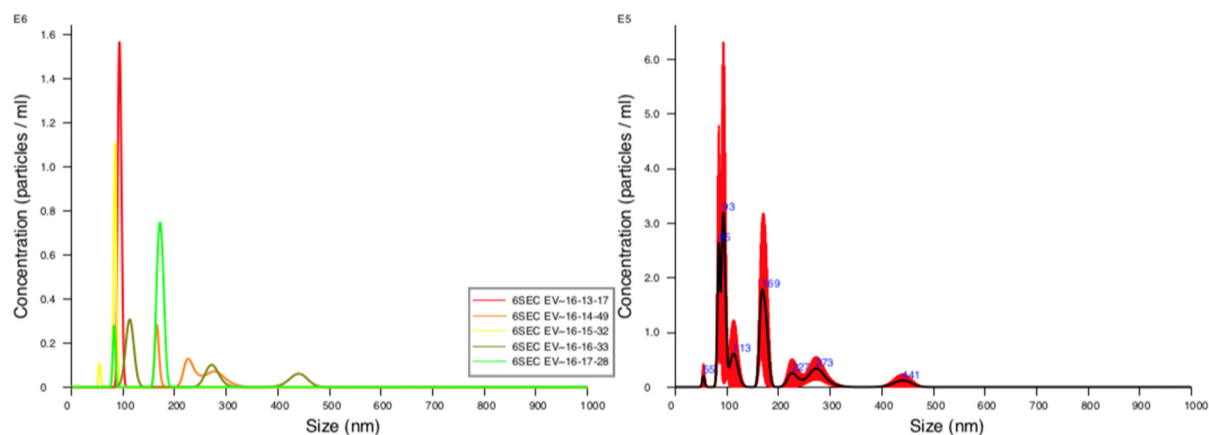


FTLA Concentration / Size graph for Experiment:
21SEC EVs 40x 25.11.20 2020-11-25 15-21-18

Averaged FTLA Concentration / Size for Experiment:
21SEC EVs 40x 25.11.20 2020-11-25 15-21-18
Error bars indicate + / -1 standard error of the mean

<p>Included Files</p> <p>21SEC EVs 40x 25.11.20 2020-11-25 15-22-17 21SEC EVs 40x 25.11.20 2020-11-25 15-23-36 21SEC EVs 40x 25.11.20 2020-11-25 15-24-22 21SEC EVs 40x 25.11.20 2020-11-25 15-25-15 21SEC EVs 40x 25.11.20 2020-11-25 15-26-00</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 03-21-18PM 25~ Time Captured: 15:21:18 25/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 21SEC EVs 40x 25.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 13 Slider Shutter: 1232 Slider Gain: 175 FPS: 25.0 Number of Frames: 749 Temperature: 23.7 - 23.9 °C Viscosity: (Water) 0.911 - 0.916 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 13.8 - 17.5 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 110.1 nm Mode: 75.9 nm SD: 46.0 nm D10: 70.8 nm D50: 95.7 nm D90: 158.3 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 110.2 +/- 4.4 nm Mode: 97.0 +/- 12.2 nm SD: 42.1 +/- 6.5 nm D10: 70.4 +/- 2.2 nm D50: 106.0 +/- 10.5 nm D90: 154.3 +/- 8.4 nm</p> <p>Concentration (Upgrade): 2.42e+08 +/- 1.77e+07 particles/ml 8.3 +/- 0.4 particles/frame 8.8 +/- 0.4 centres/frame</p>
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Figure S17: PL-Dil, SEC fraction: 5-6

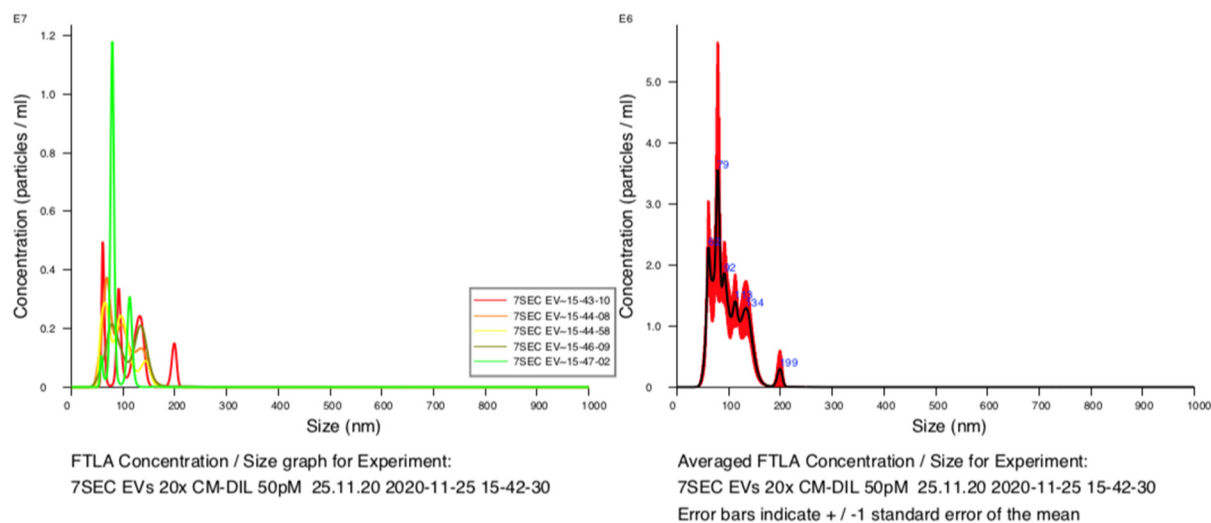


FTLA Concentration / Size graph for Experiment:
6SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-12-06

Averaged FTLA Concentration / Size for Experiment:
6SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-12-06
Error bars indicate + / - 1 standard error of the mean

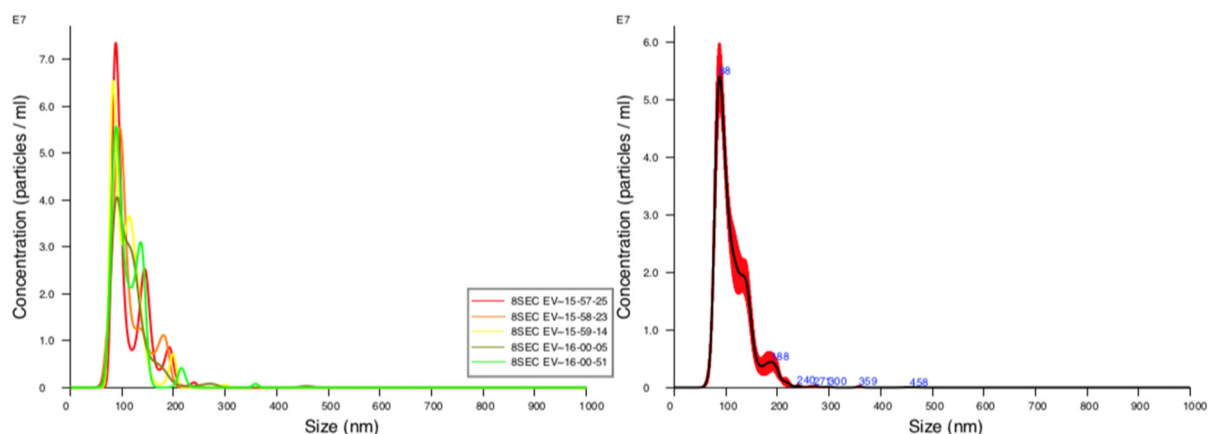
<p>Included Files</p> <p>6SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-13-17 6SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-14-49 6SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-15-32 6SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-16-33 6SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-17-28</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 04-12-06PM 25~ Time Captured: 16:12:06 25/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 6SEC EVs 20x CM-DIL 50pM 25.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 15 Slider Shutter: 1206 Slider Gain: 245 FPS: 25.0 Number of Frames: 749 Temperature: 25.1 °C Viscosity: (Water) 0.885 - 0.886 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 9.2 - 21.2 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 160.1 nm Mode: 92.9 nm SD: 88.9 nm D10: 84.7 nm D50: 123.4 nm D90: 277.0 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 158.8 +/- 31.5 nm Mode: 125.4 +/- 18.2 nm SD: 44.1 +/- 23.1 nm D10: 104.7 +/- 15.5 nm D50: 142.2 +/- 27.0 nm D90: 219.4 +/- 66.3 nm</p> <p>Concentration (Upgrade): 1.15e+07 +/- 1.23e+06 particles/ml 0.7 +/- 0.1 particles/frame 0.8 +/- 0.2 centres/frame</p> <p>Concentration measurements may be unreliable See summary file for more info</p>
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Figure S18: PL-Dil, SEC fraction: 7



<p>Included Files</p> <p>7SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 15-43-10 7SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 15-44-08 7SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 15-44-58 7SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 15-46-09 7SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 15-47-02</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 03-42-30PM 25~ Time Captured: 15:42:30 25/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 7SEC EVs 20x CM-DIL 50pM 25.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 15 Slider Shutter: 1206 Slider Gain: 245 FPS: 25.0 Number of Frames: 749 Temperature: 24.4 - 24.6 °C Viscosity: (Water) 0.897 - 0.900 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 14.9 - 19.7 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 98.2 nm Mode: 78.8 nm SD: 31.9 nm D10: 62.1 nm D50: 90.8 nm D90: 139.9 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 98.6 +/- 4.9 nm Mode: 70.3 +/- 3.6 nm SD: 29.5 +/- 4.1 nm D10: 64.6 +/- 2.6 nm D50: 95.6 +/- 6.1 nm D90: 145.6 +/- 13.2 nm Concentration (Upgrade): 1.59e+08 +/- 7.71e+06 particles/ml 8.2 +/- 0.5 particles/frame 8.5 +/- 0.5 centres/frame</p>
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Figure S19: PL-Dil, SEC fraction: 8

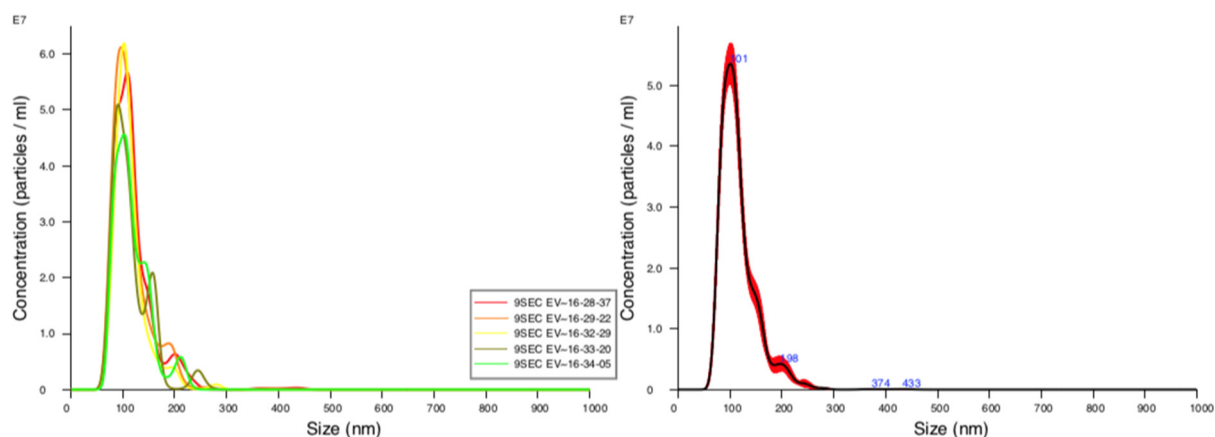


FTLA Concentration / Size graph for Experiment:
8SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 15-57-04

Averaged FTLA Concentration / Size for Experiment:
8SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 15-57-04
Error bars indicate + / - 1 standard error of the mean

<p>Included Files</p> <p>8SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 15-57-25 8SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 15-58-23 8SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 15-59-14 8SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-00-05 8SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-00-51</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 03-57-04PM 25~ Time Captured: 15:57:04 25/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 8SEC EVs 20x CM-DIL 50pM 25.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 13 Slider Shutter: 1232 Slider Gain: 175 FPS: 25.0 Number of Frames: 749 Temperature: 25.1 °C Viscosity: (Water) 0.885 - 0.886 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 14.6 - 14.9 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 112.4 nm Mode: 87.9 nm SD: 34.6 nm D10: 80.6 nm D50: 102.5 nm D90: 153.3 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 112.5 +/- 1.3 nm Mode: 89.1 +/- 2.0 nm SD: 34.5 +/- 1.6 nm D10: 80.6 +/- 0.6 nm D50: 102.5 +/- 1.3 nm D90: 154.7 +/- 5.8 nm</p> <p>Concentration (Upgrade): 2.56e+09 +/- 5.73e+07 particles/ml 119.5 +/- 2.7 particles/frame 112.6 +/- 2.1 centres/frame</p>
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Figure S20: PL-Dil, SEC fraction: 9

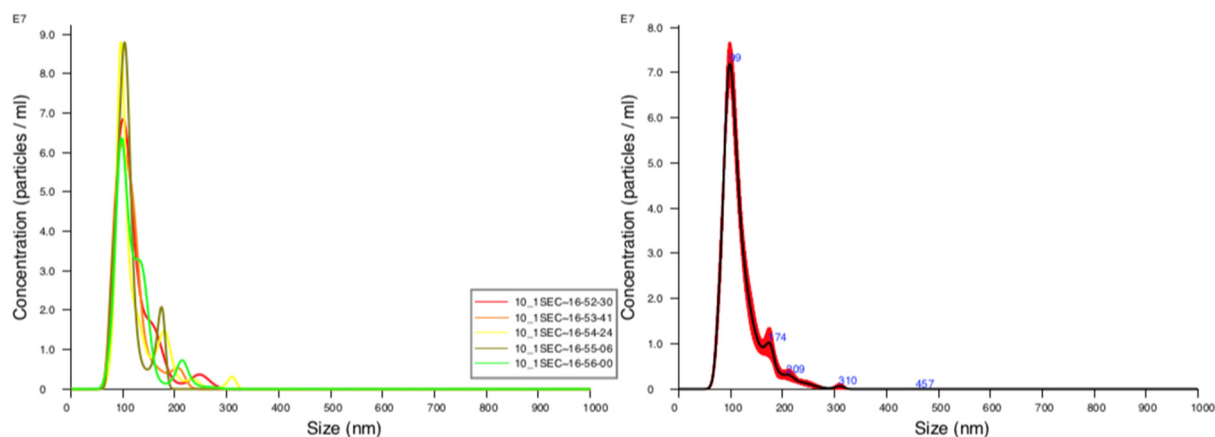


FTLA Concentration / Size graph for Experiment:
9SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-28-06

Averaged FTLA Concentration / Size for Experiment:
9SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-28-06
Error bars indicate + / -1 standard error of the mean

<p>Included Files</p> <p>9SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-28-37 9SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-29-22 9SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-32-29 9SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-33-20 9SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-34-05</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 04-28-06PM 25~ Time Captured: 16:28:06 25/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 9SEC EVs 20x CM-DIL 50pM 25.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 13 Slider Shutter: 1232 Slider Gain: 175 FPS: 25.0 Number of Frames: 749 Temperature: 25.4 °C Viscosity: (Water) 0.879 - 0.880 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 14.1 - 15.0 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 116.2 nm Mode: 100.7 nm SD: 37.5 nm D10: 80.5 nm D50: 107.6 nm D90: 161.5 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 116.1 +/- 1.1 nm Mode: 100.1 +/- 3.1 nm SD: 37.0 +/- 1.8 nm D10: 80.6 +/- 0.8 nm D50: 107.6 +/- 1.0 nm D90: 161.4 +/- 2.8 nm</p> <p>Concentration (Upgrade): 3.38e+09 +/- 1.54e+08 particles/ml 175.5 +/- 10.7 particles/frame 151.2 +/- 6.9 centres/frame</p>
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Figure S21: PL-Dil, SEC fraction: 10

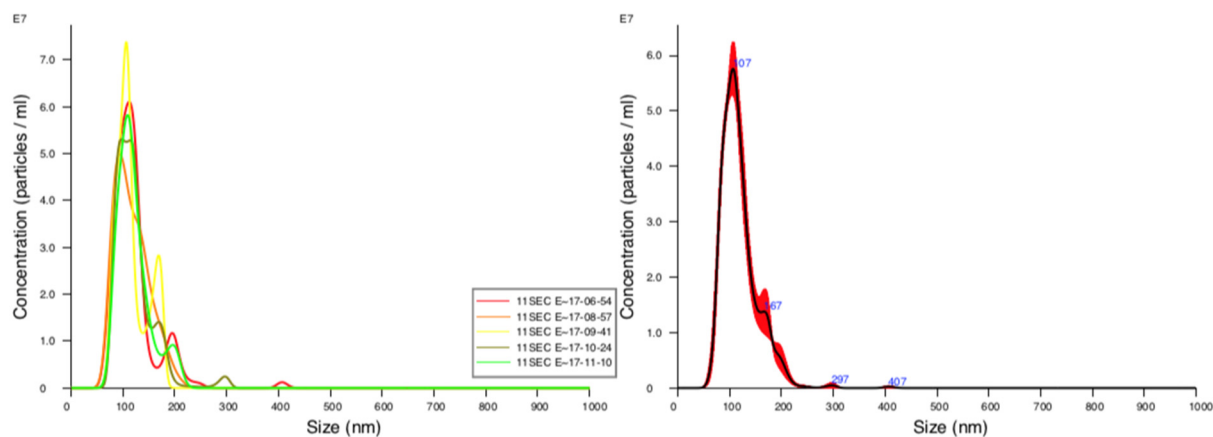


FTLA Concentration / Size graph for Experiment:
10_1SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-51-43

Averaged FTLA Concentration / Size for Experiment:
10_1SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-51-43
Error bars indicate + / - 1 standard error of the mean

<p>Included Files</p> <p>10_1SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-52-30 10_1SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-53-41 10_1SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-54-24 10_1SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-55-06 10_1SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 16-56-00</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 04-51-43PM 25~ Time Captured: 16:51:43 25/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 10_1SEC EVs 20x CM-DIL 50pM 25.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 12 Slider Shutter: 1200 Slider Gain: 125 FPS: 25.0 Number of Frames: 749 Temperature: 25.6 - 25.7 °C Viscosity: (Water) 0.876 - 0.877 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 14.2 - 14.7 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 117.8 nm Mode: 98.3 nm SD: 36.9 nm D10: 84.5 nm D50: 107.2 nm D90: 168.9 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 117.8 +/- 2.4 nm Mode: 99.0 +/- 1.3 nm SD: 36.1 +/- 3.2 nm D10: 84.5 +/- 0.9 nm D50: 107.5 +/- 1.2 nm D90: 165.9 +/- 5.5 nm</p> <p>Concentration (Upgrade): 3.58e+09 +/- 1.00e+08 particles/ml 140.6 +/- 6.5 particles/frame 134.0 +/- 4.8 centres/frame</p>
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Figure S22: PL-Dil, SEC fraction: 11

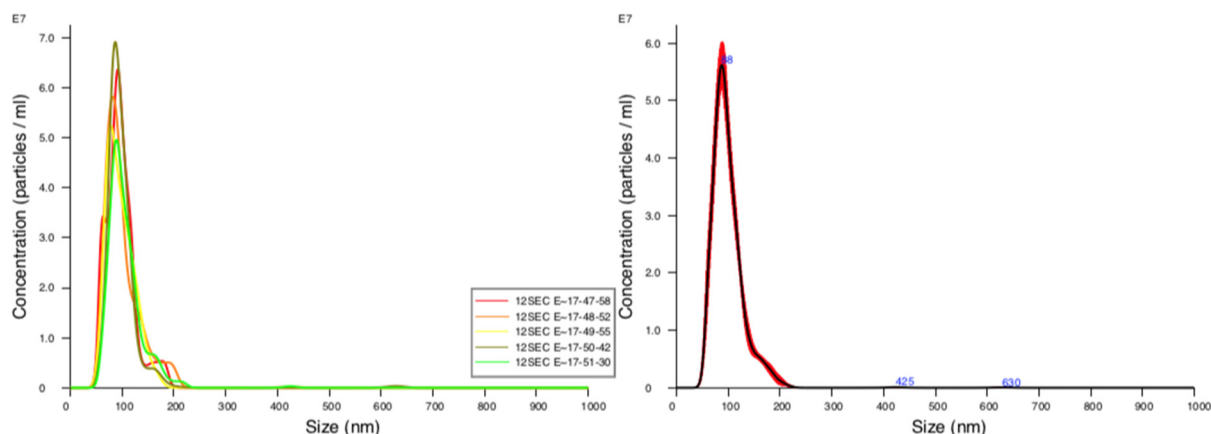


FTLA Concentration / Size graph for Experiment:
11SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 17-06-39

Averaged FTLA Concentration / Size for Experiment:
11SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 17-06-39
Error bars indicate + / -1 standard error of the mean

<p>Included Files</p> <p>11SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 17-06-54 11SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 17-08-57 11SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 17-09-41 11SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 17-10-24 11SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 17-11-10</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 05-06-39PM 25~ Time Captured: 17:06:39 25/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 11SEC EVs 20x CM-DIL 50pM 25.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 12 Slider Shutter: 1200 Slider Gain: 125 FPS: 25.0 Number of Frames: 749 Temperature: 25.1 - 25.2 °C Viscosity: (Water) 0.884 - 0.887 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 14.3 - 14.7 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 119.2 nm Mode: 106.5 nm SD: 36.1 nm D10: 82.5 nm D50: 111.8 nm D90: 168.4 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 119.2 +/- 0.9 nm Mode: 103.7 +/- 3.5 nm SD: 35.5 +/- 2.5 nm D10: 82.7 +/- 1.5 nm D50: 112.0 +/- 1.0 nm D90: 170.7 +/- 3.1 nm</p> <p>Concentration (Upgrade): 3.76e+09 +/- 8.95e+07 particles/ml 148.3 +/- 4.6 particles/frame 147.7 +/- 4.8 centres/frame</p>
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Figure S23: PL-Dil, SEC fraction: 12

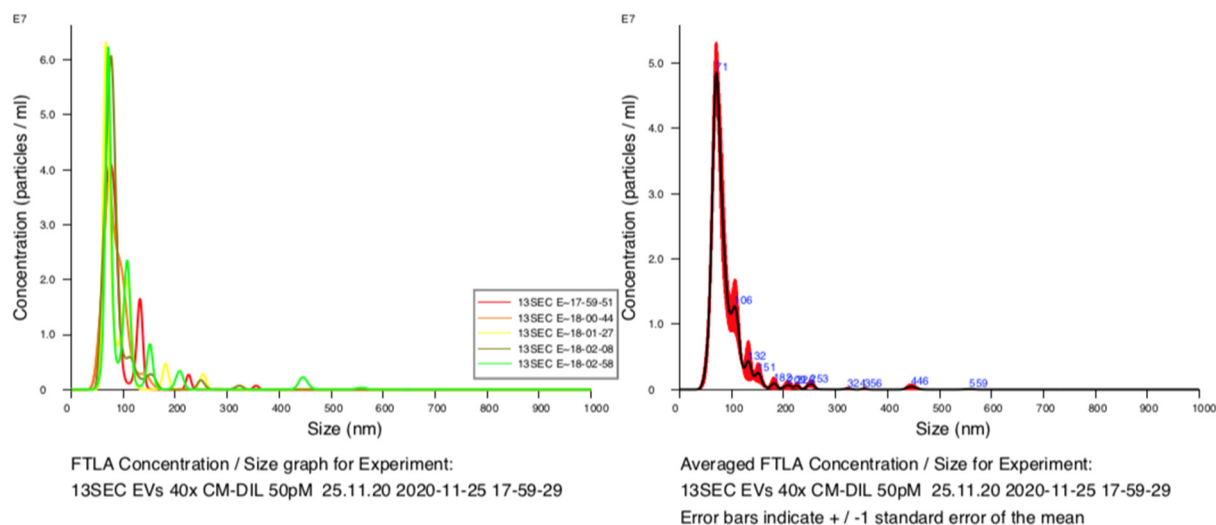


FTLA Concentration / Size graph for Experiment:
 12SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 17-47-36

Averaged FTLA Concentration / Size for Experiment:
 12SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 17-47-36
 Error bars indicate + / - 1 standard error of the mean

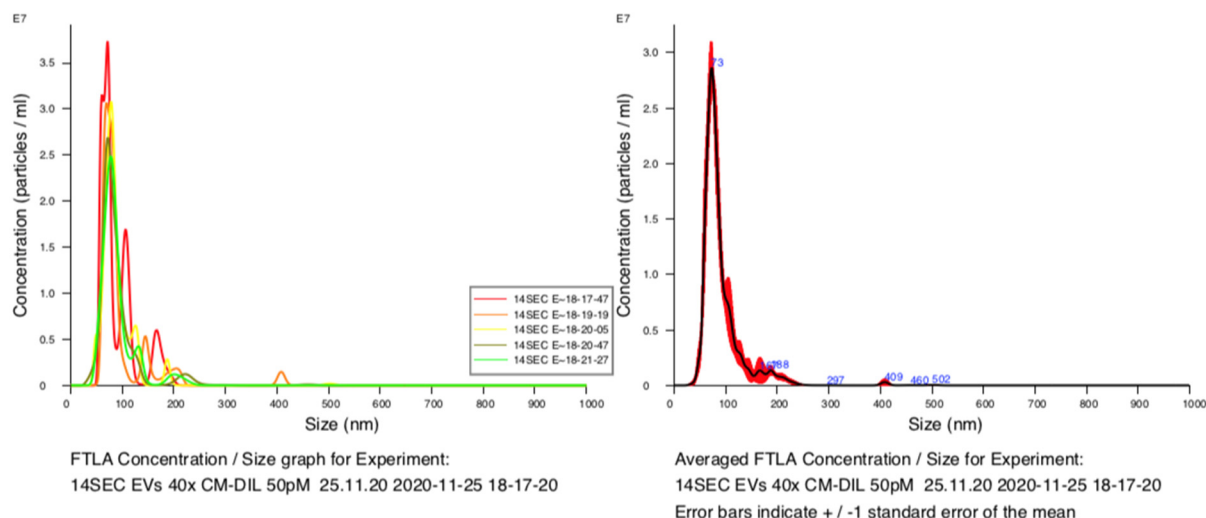
<p>Included Files</p> <p>12SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 17-47-58 12SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 17-48-52 12SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 17-49-55 12SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 17-50-42 12SEC EVs 20x CM-DIL 50pM 25.11.20 2020-11-25 17-51-30</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 05-47-36PM 25~ Time Captured: 17:47:36 25/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 12SEC EVs 20x CM-DIL 50pM 25.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 13 Slider Shutter: 1232 Slider Gain: 175 FPS: 25.0 Number of Frames: 749 Temperature: 25.2 - 25.3 °C Viscosity: (Water) 0.882 - 0.884 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 15.3 - 16.1 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 99.8 nm Mode: 87.6 nm SD: 35.7 nm D10: 67.6 nm D50: 93.5 nm D90: 136.9 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 100.0 +/- 1.6 nm Mode: 85.6 +/- 2.4 nm SD: 35.0 +/- 3.4 nm D10: 68.0 +/- 1.8 nm D50: 93.6 +/- 1.2 nm D90: 137.0 +/- 5.0 nm</p> <p>Concentration (Upgrade): 3.15e+09 +/- 1.08e+08 particles/ml 114.9 +/- 5.8 particles/frame 122.7 +/- 6.0 centres/frame</p>
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Figure S24: PL-Dil, SEC fraction: 13



<p>Included Files</p> <p>13SEC EVs 40x CM-DIL 50pM 25.11.20 2020-11-25 17-59-51 13SEC EVs 40x CM-DIL 50pM 25.11.20 2020-11-25 18-00-44 13SEC EVs 40x CM-DIL 50pM 25.11.20 2020-11-25 18-01-27 13SEC EVs 40x CM-DIL 50pM 25.11.20 2020-11-25 18-02-08 13SEC EVs 40x CM-DIL 50pM 25.11.20 2020-11-25 18-02-58</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 05-59-29PM 25~ Time Captured: 17:59:29 25/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 13SEC EVs 40x CM-DIL 50pM 25.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 14 Slider Shutter: 1259 Slider Gain: 245 FPS: 25.0 Number of Frames: 749 Temperature: 25.2 - 25.3 °C Viscosity: (Water) 0.883 - 0.885 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 16.5 - 17.5 pix</p>	<p>Results</p> <p>Stats: Merged Data Mean: 90.5 nm Mode: 70.6 nm SD: 46.6 nm D10: 61.9 nm D50: 77.6 nm D90: 126.0 nm</p> <p>Stats: Mean +/- Standard Error Mean: 90.8 +/- 4.1 nm Mode: 72.2 +/- 1.6 nm SD: 42.7 +/- 9.1 nm D10: 61.7 +/- 1.1 nm D50: 77.5 +/- 1.1 nm D90: 125.7 +/- 7.9 nm</p> <p>Concentration (Upgrade): 1.65e+09 +/- 5.05e+07 particles/ml 51.7 +/- 0.7 particles/frame 60.3 +/- 1.0 centres/frame</p>
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Figure S25: PL-Dil, SEC fraction: 14



Included Files

14SEC EVs 40x CM-DIL 50pM 25.11.20 2020-11-25 18-17-47
14SEC EVs 40x CM-DIL 50pM 25.11.20 2020-11-25 18-19-19
14SEC EVs 40x CM-DIL 50pM 25.11.20 2020-11-25 18-20-05
14SEC EVs 40x CM-DIL 50pM 25.11.20 2020-11-25 18-20-47
14SEC EVs 40x CM-DIL 50pM 25.11.20 2020-11-25 18-21-27

Details

NTA Version: NTA 3.4 Build 3.4.003
Script Used: SOP Standard Measurement 06-17-20PM 25~
Time Captured: 18:17:20 25/11/2020
Operator: Nadya24.11.20
Pre-treatment:
Sample Name: 14SEC EVs 40x CM-DIL 50pM 25.11.20
Diluent: PBS
Remarks:

Capture Settings

Camera Type: sCMOS
Laser Type: Blue488
Camera Level: 14
Slider Shutter: 1259
Slider Gain: 245
FPS: 25.0
Number of Frames: 749
Temperature: 25.0 - 25.1 °C
Viscosity: (Water) 0.887 - 0.889 cP
Dilution factor: Dilution not recorded

Analysis Settings

Detect Threshold: 17
Blur Size: Auto
Max Jump Distance: Auto: 16.0 - 17.6 pix

Results

Stats: Merged Data

Mean: 91.0 nm
Mode: 72.7 nm
SD: 43.4 nm
D10: 60.6 nm
D50: 78.8 nm
D90: 132.4 nm

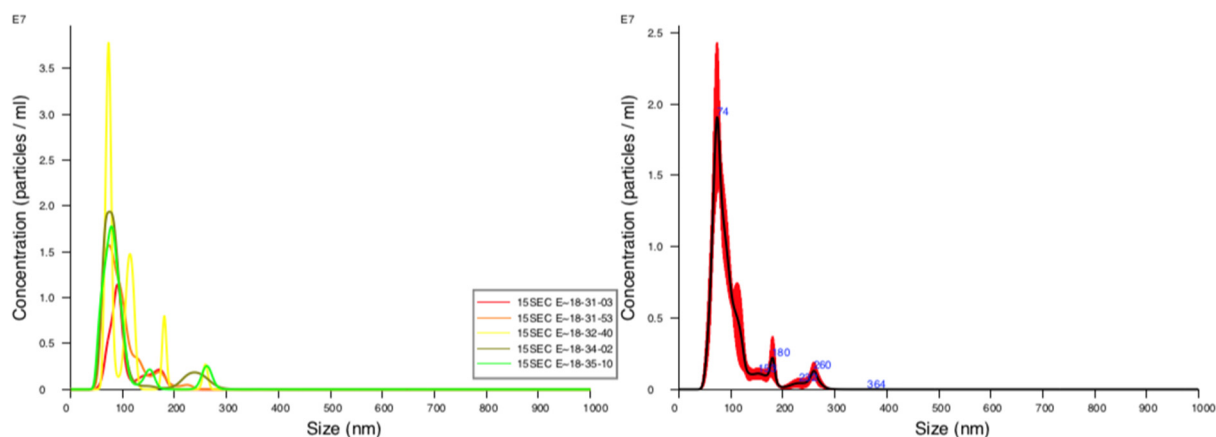
Stats: Mean +/- Standard Error

Mean: 91.0 +/- 1.9 nm
Mode: 73.8 +/- 1.8 nm
SD: 42.4 +/- 4.9 nm
D10: 60.7 +/- 0.8 nm
D50: 78.7 +/- 1.0 nm
D90: 137.6 +/- 8.2 nm

Concentration (Upgrade): 1.14e+09 +/- 4.77e+07 particles/ml
37.8 +/- 2.4 particles/frame
44.4 +/- 2.6 centres/frame

Concentration measurements may require some caution due to noise
See summary file for more info

Figure S26: PL-Dil, SEC fraction: 15

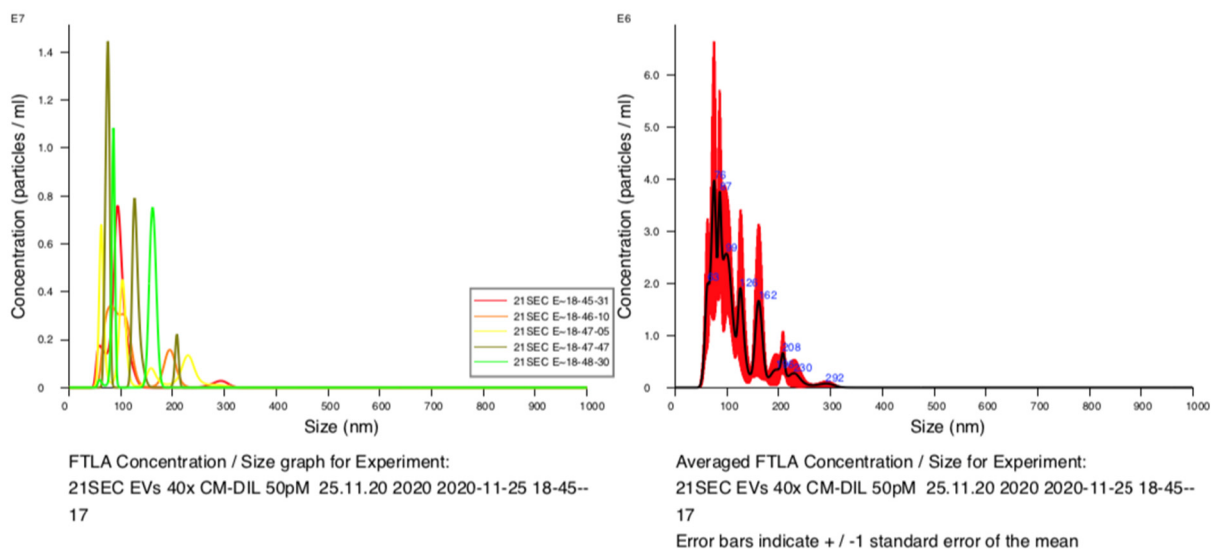


FTLA Concentration / Size graph for Experiment:
 15SEC EVs 40x CM-DIL 50pM 25.11.20 2020-11-25 18-30-29

Averaged FTLA Concentration / Size for Experiment:
 15SEC EVs 40x CM-DIL 50pM 25.11.20 2020-11-25 18-30-29
 Error bars indicate + / - 1 standard error of the mean

<p>Included Files</p> <p>15SEC EVs 40x CM-DIL 50pM 25.11.20 2020-11-25 18-31-03 15SEC EVs 40x CM-DIL 50pM 25.11.20 2020-11-25 18-31-53 15SEC EVs 40x CM-DIL 50pM 25.11.20 2020-11-25 18-32-40 15SEC EVs 40x CM-DIL 50pM 25.11.20 2020-11-25 18-34-02 15SEC EVs 40x CM-DIL 50pM 25.11.20 2020-11-25 18-35-10</p> <p>Details</p> <p>NTA Version: NTA 3.4 Build 3.4.003 Script Used: SOP Standard Measurement 06-30-29PM 25~ Time Captured: 18:30:29 25/11/2020 Operator: Nadya24.11.20 Pre-treatment: Sample Name: 15SEC EVs 40x CM-DIL 50pM 25.11.20 Diluent: PBS Remarks:</p> <p>Capture Settings</p> <p>Camera Type: sCMOS Laser Type: Blue488 Camera Level: 14 Slider Shutter: 1259 Slider Gain: 245 FPS: 25.0 Number of Frames: 749 Temperature: 25.0 - 25.1 °C Viscosity: (Water) 0.886 - 0.889 cP Dilution factor: Dilution not recorded</p> <p>Analysis Settings</p> <p>Detect Threshold: 17 Blur Size: Auto Max Jump Distance: Auto: 15.7 - 16.8 pix</p>	<p>Results</p> <p>Stats: Merged Data</p> <p>Mean: 99.9 nm Mode: 73.6 nm SD: 46.5 nm D10: 64.2 nm D50: 84.3 nm D90: 165.1 nm</p> <p>Stats: Mean +/- Standard Error</p> <p>Mean: 100.2 +/- 1.3 nm Mode: 78.2 +/- 3.2 nm SD: 44.4 +/- 4.7 nm D10: 65.0 +/- 2.2 nm D50: 84.7 +/- 2.3 nm D90: 172.8 +/- 13.1 nm</p> <p>Concentration (Upgrade): 8.02e+08 +/- 9.02e+07 particles/ml 28.7 +/- 3.1 particles/frame 31.8 +/- 3.5 centres/frame</p>
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Figure S27: PL-Dil, SEC fraction: 16-21



Included Files	Results
21SEC EVs 40x CM-DIL 50pM 25.11.20 2020 2020-11-25 18-45-31	Stats: Merged Data
21SEC EVs 40x CM-DIL 50pM 25.11.20 2020 2020-11-25 18-46-10	Mean: 114.3 nm
21SEC EVs 40x CM-DIL 50pM 25.11.20 2020 2020-11-25 18-47-05	Mode: 75.1 nm
21SEC EVs 40x CM-DIL 50pM 25.11.20 2020 2020-11-25 18-47-47	SD: 48.7 nm
21SEC EVs 40x CM-DIL 50pM 25.11.20 2020 2020-11-25 18-48-30	D10: 67.9 nm
	D50: 98.8 nm
	D90: 185.3 nm
Details	Stats: Mean +/- Standard Error
NTA Version: NTA 3.4 Build 3.4.003	Mean: 115.1 +/- 5.6 nm
Script Used: SOP Standard Measurement 06-45-17PM 25--	Mode: 79.5 +/- 5.3 nm
Time Captured: 18:45:17 25/11/2020	SD: 46.6 +/- 5.4 nm
Operator: Nadya24.11.20	D10: 70.1 +/- 3.7 nm
Pre-treatment:	D50: 106.5 +/- 12.7 nm
Sample Name: 21SEC EVs 40x CM-DIL 50pM 25.11.20 2020	D90: 170.8 +/- 20.5 nm
Diluent: PBS	Concentration (Upgrade): 2.34e+08 +/- 1.14e+07 particles/ml
Remarks:	8.7 +/- 0.1 particles/frame
	9.5 +/- 0.2 centres/frame
Capture Settings	
Camera Type: sCMOS	
Laser Type: Blue488	
Camera Level: 13	
Slider Shutter: 1232	
Slider Gain: 175	
FPS: 25.0	
Number of Frames: 749	
Temperature: 24.6 - 24.8 °C	
Viscosity: (Water) 0.893 - 0.896 cP	
Dilution factor: Dilution not recorded	
Analysis Settings	
Detect Threshold: 17	
Blur Size: Auto	
Max Jump Distance: Auto: 14.1 - 23.7 pix	