Adenoviral Reference Material Working Group
Bid Submission Form
Electron Microscopy Methods Description

EM METHOD: Evaluation of Singlets/Duplets/Multplets at Various Dilutions in Buffer

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Materials:

1. Samples including positive control (aged production batch) and negative control (formulation blank)
2. Formvar carbon 200 mesh copper grids, Ted Pella
3. Glutaraldehyde, 50%, Ted Pella.
4. 1.5% Glutaraldehyde in 1X DPBS, pH 7.6, 460 mOs/kg. Dilute 50% Glutaraldehyde stock to 1.5% in 1X DPBS (150 µL Glutaraldehyde stock plus 4850 µL 1X PBS)
5. Polished distilled water, Millipore
6. 10X DPBS, Sigma
7. 1X DPBS, pH 7.6, prepared from 10X DPBS, 1:10 dilution in polished, distilled water
8. Conductive carbon tabs, Ted Pella, cat. no. 16084-1
9. Conducting ink pen, Ted Pella, cat. no. 16044
10. Aluminum metal stubs, Ted Pella (12.5 mm x 10 mm)
11. Uranyl Acetate, Ted Pella
12. Fine forceps
13. Kimwipes

Procedure:

NOTE: Latex gloves must be worn throughout the procedure.

1. Label metal stubs, 1 through 8. Overlay each stub with one conductive carbon tab using a fine forcep.
2. Adhere one grid onto each stub surface.
3. Prepare dilution series on each sample (positive/negative) as follows:

Dilution	Sample (µL)	GFB Buffer (µL)
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1:2	50	50
1:5	20	80
1:10	10	90

4. Pipet 20 µL sample onto grid surface.
5. Incubate for 2 minutes to permit adsorption of the sample on the substrate surface.
6. Remove excess sample and other contaminants on each grid by touching a filter paper or kimwipe to the edge of each grid surface.

7. Fix each sample (including the grid only control) with 1.5% glutaraldehyde/DPBS for 5 minutes.
8. Remove fixative using filter paper or kimwipe as in step 6 above.
9. Wash each grid with 1X DPBS for 1 minute followed by two washes in polished, distilled water, 30 seconds in duration each wash.
10. Prepare 1% uranyl acetate (UA) as follows: 1.0 g in 100 mL polished, distilled water.
11. Stain all samples with 1% UA solution for 10 minutes at room temperature.
12. Wash each grid four times with polished, distilled water (30 seconds each wash).
13. Store samples dry at room temperature in a vacuum dessicator under the laminar flow hood.
14. View samples on the FESEM using secondary electron imaging. Take images across the field.
15. Analyze samples using the adenovirus IPP macro.

EM METHOD: Evaluation of Particle Concentration

Procedure:

1. Prepare samples as described in ***Evaluation of Singlets/Duplets/Multplets at Various Dilutions in Buffer.***
2. Upon viewing samples on grids using the FESEM using secondary electron imaging, count particle numbers as per hemacytometer counting of cells.
3. Count a statistically appropriate number of grid sections (typically 100).
4. Calculation must include dilution factor.
5. Verify sample integrity as part of data review.