

Accurate mass calibration with MassFERENCE™ P2

MassFERENCE P2 is a mass photometry calibrant, recommended for use with the SamuxMP and SamuxMP Auto for AAV sample characterization. MassFERENCE P2 can be reliably used as a single-point calibrant for AAV samples with particles in the 670 kDa–5 MDa mass range.

MassFERENCE P2: A calibrant for AAV analytics

Mass photometry measures the interference between the light scattered by individual biomolecules in a sample and the light reflected by the measurement surface. As this signal – known as “contrast” – scales with mass in a linear fashion¹, it is possible to use it to calculate the mass of the biomolecules in the sample by using molecules of known mass (calibrants) as a reference.

With the aim to streamline AAV characterization, Refeyn provides MassFERENCE P2, a bespoke calibrant for the SamuxMP and SamuxMP Auto mass photometers that was carefully selected for the stability and accuracy of calibration it provides to mass photometry users.

MassFERENCE P2 contains a standard protein species of 3.64 MDa, tailored to calibrate mass photometry measurements of AAV-containing samples with masses ranging from 670 kDa to 5 MDa. The mass of MassFERENCE P2 was verified using mass spectrometry.

Calibrations using this single point are reliable thanks to the linearity of the contrast-mass relationship throughout the working mass range¹.

MassFERENCE P2 calibrations are precise

To test the repeatability of calibrations performed with MassFERENCE P2, we first used it for calibration (Fig. 1), which was then applied to three measurements of an empty AAV8 sample (Fig. 2).

The average measured mass was 3.743 ± 0.002 MDa, which is within the 2% precision specified for the Samux™ line of instruments and in line with the 3.7–3.8 MDa estimated for empty AAV8 capsids.

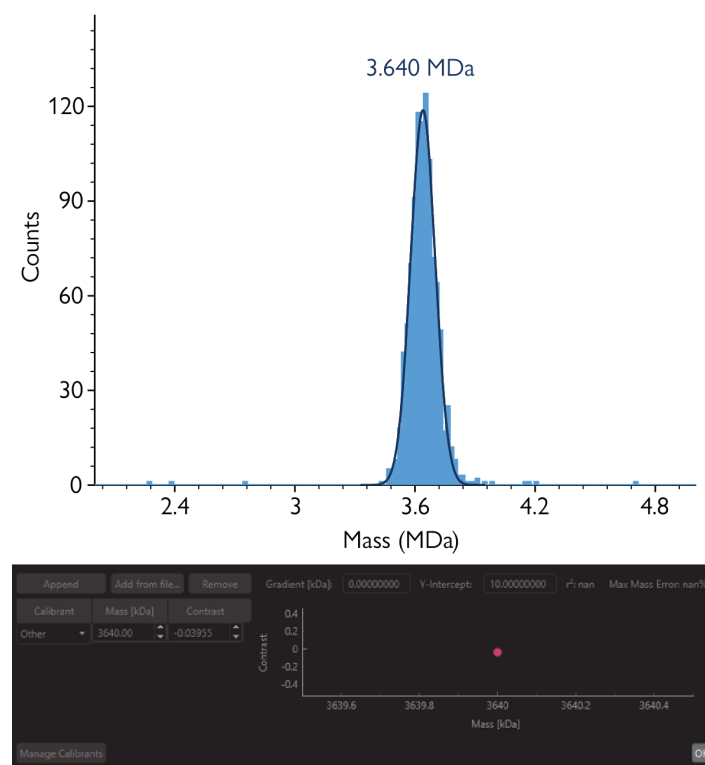


Figure 1. A mass photometry measurement of MassFERENCE P2. The top panel shows that MassFERENCE P2 presents as a single peak at 3.64 MDa. The bottom panel shows the interface where the calibrant mass is assigned to the measured contrast value, establishing a contrast-mass relationship. This calibration was applied to all other measurements shown in this tech note. Measured using the SamuxMP Auto.

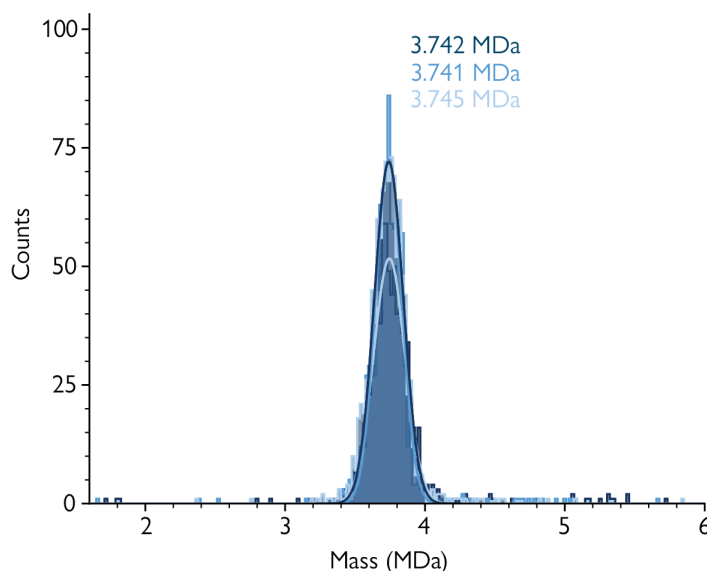


Figure 2. AAV measurements calibrated with MassFERENCE P2 show high precision. The mass histogram shows three independent measurements of the same AAV8 sample. Between-measurement variability was low, with an average measured mass of 3.743 ± 0.002 MDa, consistent with the estimated masses of empty AAV8 capsids. The MassFERENCE P2 calibration (Fig. 1) was applied to all three measurements. Measured using the SamuxMP Auto.

MassFERENCE P2 calibrations are accurate

We used a MassFERENCE P2 calibration (Fig. 1) when measuring a mixed sample containing empty AAV8 capsids (3.7 MDa) and cross-linked thyroglobulin (TG) monomers (0.67 MDa) and dimers (1.34 MDa). The average mass measured for the AAV8 was within the estimated mass for empty capsids (3.7–3.8 MDa). The average masses measured for TG monomers and dimers were within 5% of the known masses, consistent with the accuracy of the SamuxMP. The mass precision was within the 2% instrument limit for all components of the sample (Fig. 3).

One-point calibration with MassFERENCE P2 is reliable

To test the reliability of one-point calibration with MassFERENCE P2, we compared mass photometry measurements calibrated with one-point (MassFERENCE P2), two-point (mix of MassFERENCE P2 and TG) or three-point (cross-linked TG) calibrations. All three approaches gave a linear contrast-mass relationship (Fig. 4) and highly similar results (Table 1). The mass measured for the AAV6 sample with two- and three-point calibrations differed from that of the single-point approach by less than 0.6%.

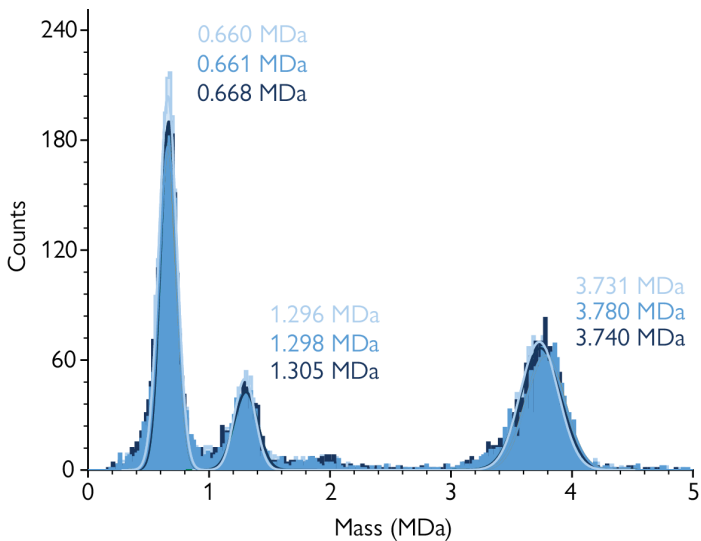


Figure 3. MassFERENCE P2 provides accurate mass calibrations across a wide mass range. The mass photometry histogram shows the measurement of a mixed sample of AAV8 and cross-linked thyroglobulin (TG). The first two peaks correspond to TG monomers (0.67 MDa) and dimers (1.34 MDa). The third peak corresponds to empty AAV8 empty capsids (estimated mass: 3.7–3.8 MDa). Measurement calibrated with the MassFERENCE P2 calibration (Fig. 1). Measured on the SamuxMP Auto.

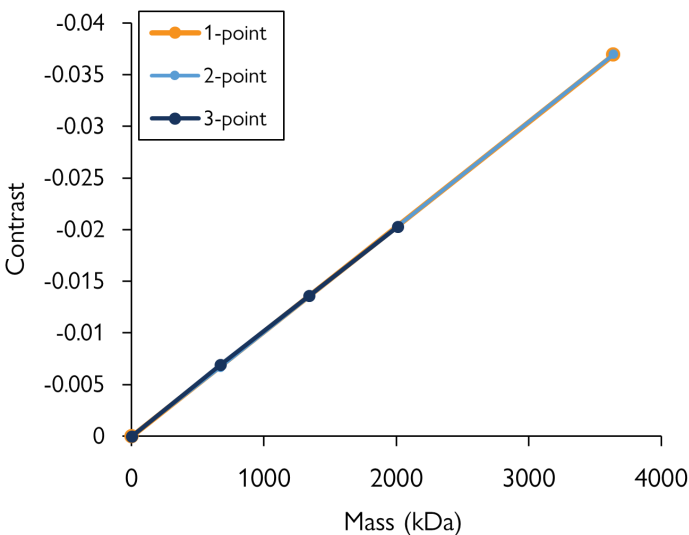


Figure 4. The contrast-mass relationship is linear and similar for one-, two- and three-point calibrations. The results of applying each of these calibrations to the same AAV6 sample are shown in Table 1.

Table 1. Mass measurements of a sample of empty AAV6 capsids applying one-point (MassFERENCE P2), two-point (MassFERENCE P2+TG) and three-point (cross-linked TG) calibrations. Four replicates were taken for each measurement. The bottom row (Difference (%)) compares the average mass measured with two- and three-point calibrations to that measured with one-point (MassFERENCE P2) calibration.

Calibration/ Replicates	One-point	Two-point	Three-point
1	3731	3738	3793
2	3733	3724	3752
3	3734	3773	3785
4	3818	3731	3771
Average (kDa)	3754	3742	3775
Standard deviation	42	22	18
CV (%)	1.13	0.58	0.47
Difference (%)	N/A	0.33	0.57

Conclusions

- Measurements calibrated with MassFERENCE P2 have high accuracy, with errors within 5% of known mass
- The mass precision of these measurements is within the 2% instrument limit in the 670 kDa–5 MDa range
- MassFERENCE P2 provides accurate and precise single-point calibrations within the relevant mass range

References

[†]Young et al., Science. 2018