

CALCULATION OF RESULTS

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve (4PLC) fit. The sample concentrations obtained should be multiplied by the dilution factor to obtain neat sample values.

Or use the online tool from MyAssays to calculate the data:

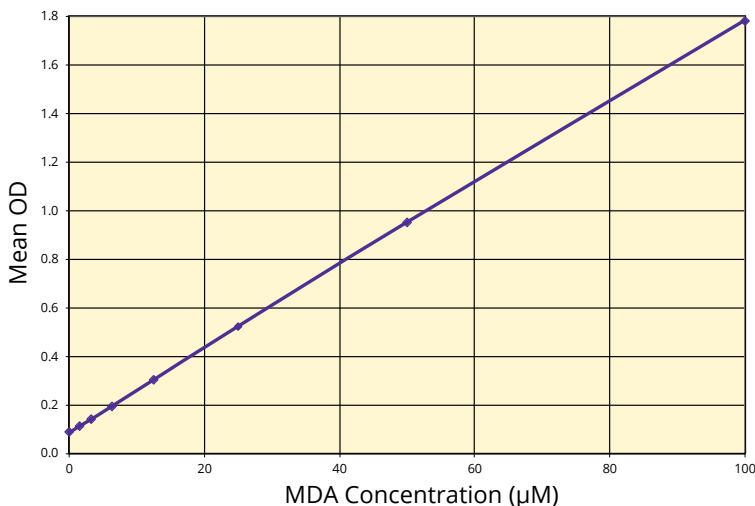
<https://www.myassays.com/arbor-assays-detectx-tbars-md-a-universal-colorimetric-detection-kit-k077.assay>

TYPICAL DATA

| Sample | Mean OD | TBARS/MDA Conc. (μM) |
|------------|---------|-----------------------------------|
| Zero | 0.087 | 0 |
| Standard 1 | 1.783 | 100 |
| Standard 2 | 0.953 | 50 |
| Standard 3 | 0.525 | 25 |
| Standard 4 | 0.305 | 12.5 |
| Standard 5 | 0.195 | 6.25 |
| Standard 6 | 0.140 | 3.125 |
| Standard 7 | 0.115 | 1.563 |
| Sample 1 | 0.537 | 25.79 |
| Sample 2 | 0.939 | 49.14 |

Always run your own standard curves for calculation of results. Do not use this data.

Typical Standard Curve



Always run your own standard curves for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the ODs for twenty wells run for each of the zero and standard #7. The detection limit was determined at two (2) standard deviations from the zero along the standard curve.

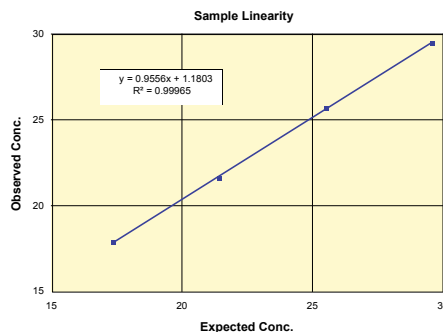
Sensitivity was determined as 0.36 μM .

The Limit of Detection was determined in a similar manner by comparing the ODs for twenty wells run for each of the zero and a low concentration sample. **The Limit of Detection was determined as 0.620 μM .**

Linearity

Linearity was determined by taking two rat serum samples with known MDA concentrations and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

| High | Low | Expected Conc. (μM) | Observed Conc. (μM) | % Recovery |
|------|-----|----------------------------------|----------------------------------|---------------|
| 80% | 20% | 29.62 | 29.46 | 99.5 |
| 60% | 40% | 25.55 | 25.67 | 100.5 |
| 40% | 60% | 21.47 | 21.56 | 100.4 |
| 20% | 80% | 17.40 | 17.84 | 102.5 |
| | | | Mean Recovery | 100.7% |



Intra Assay Precision

Three samples were run in replicates of 16 in an assay. The mean and precision of the calculated concentrations were:

| Sample | MDA Conc. (μM) | %CV |
|--------|-----------------------------|------|
| 1 | 23.5 | 12.9 |
| 2 | 48.9 | 3.5 |
| 3 | 67.1 | 12.7 |

Inter Assay Precision

Three samples were run in duplicate in 20 assays run over multiple days by several operators. The mean and precision of the calculated concentrations were:

| Sample | MDA Conc. (μM) | %CV |
|--------|-----------------------------|------|
| 1 | 23.4 | 13.8 |
| 2 | 48.3 | 4.5 |
| 3 | 68.3 | 11.6 |



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Arbor Assays and the International Society of Wildlife Endocrinology (ISWE) signed an exclusive agreement for Arbor Assays to supply ISWE members with assay kits and reagents for wildlife conservation research.

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