Biomedal

AlerTox ELISA Kits

Spiking protocol for the AlerTox ELISA Casein
(Appendix to the instruction for use)

96 Well ELISA

Order No KT-5761

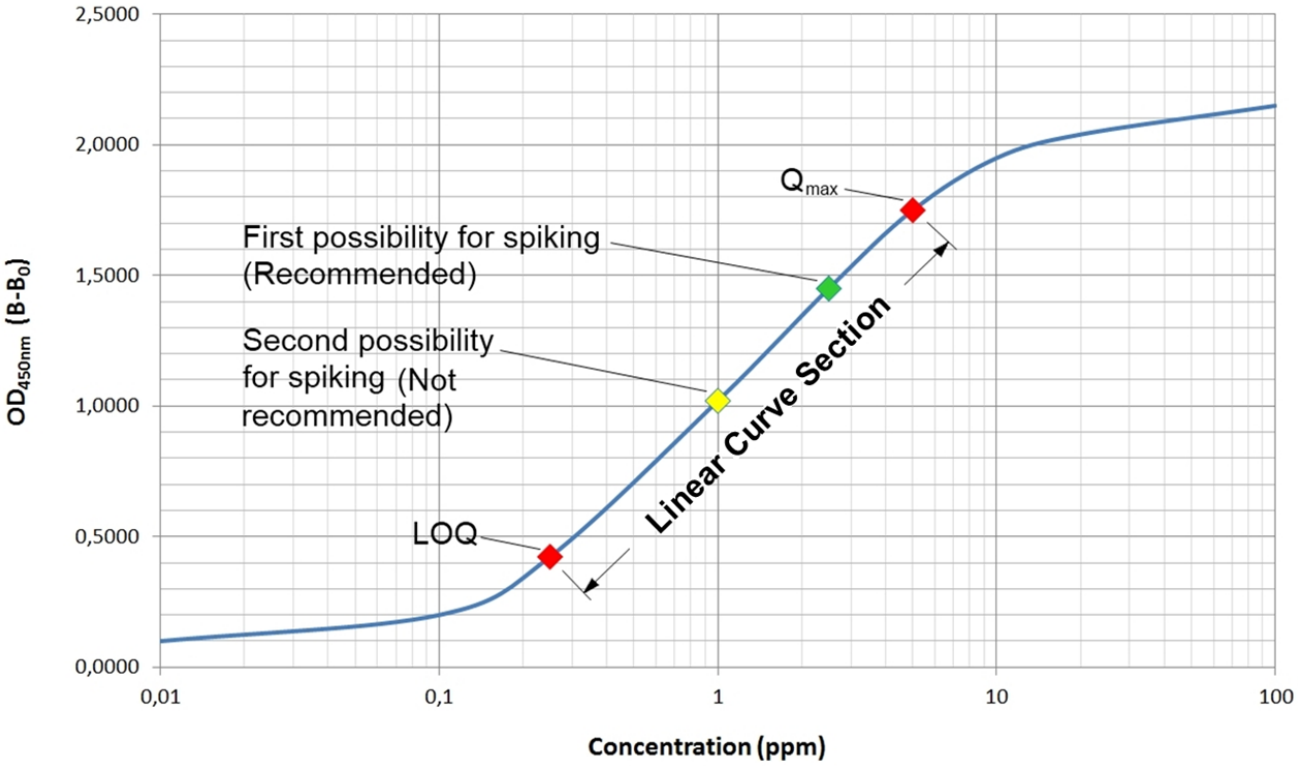
In vitro test
Storage: 2–8°C

1. The Standard Curve

The standard curve shown below is a typically semi-logarithmic curve for sandwich ELISA, being adapted to the quantitation range of the AlerTox ELISA kits. This curve is only an example and shouldn't be used for calculation, because the shape of the curve is idealized and will additionally vary from lot to lot.

When you are using reader-specific calculation software, please switch-off the 10% extrapolation option in both directions. Program the software to do a B-Bo calculation.
If the software does not allow this, please define the zero-standard as blank in A1 and A2. Pipette the 0.25 ppm followed by the other standards directly behind the blank starting with B1,2 until E1,2. Please do not pipette the zero-stand once as blank and again once as the first standard. If you would do so, the zero-standard as the first standard will be measured with an OD-value of 0.00 with the consequence, that you force the software to use 0 ppm as point of curve. This will influence the curve geometry and you will get wrong results. When you look at the curve below, you see a linear section between LOQ and Q_{max}, and non-linear parts of the curve below LOQ and above Q_{max}. Please measure always only in the linear section. In the non-linear parts of the curve you cannot measure precisely enough.

All calculated values being at least below LOQ have to be regarded as being negative, except values for those samples, which you have additionally diluted. These results, when being between LOD and LOQ are regarded as doubtful negative results. Please repeat the measurement of these samples without the additional dilution.



2. Spiking Samples

We ask you, neither to change the standard values, nor to add additional standards by diluting higher ones with the target to drop the 5 ppm standard. This can have an influence on the shape of the curve and on the formula and results in values being different to those from the original curve.

The standard range – equal with the quantification range – starts with the LOQ at 0.25 ppm and ends with the highest standard Q_{max} at 5 ppm (the 2 red points in the curve). The best linearity you always have exactly in middle of the standard range.That is the position, where the spiked sample has to be. In this particular case the spike sample should have a concentration of 2.5 ppm (green point of the curve). This is the concentration we recommend.

The yellow point seems to be in the middle of the curve, not really being. Please do not oversee the logarithmic spread. You can use 1 ppm also, but we do not recommend.

Preparation of a sample with 2.5 ppm Casein (recommended):

1.) Add 50 µL of the 500 ppm Spike Solution (undiluted 5 ppm standard) to 500 µL of wine and mixed thoroughly.
2.) Now add 9,450 µL of pre-diluted sample buffer to the spiked wine and mix again. The dilution factor is 1:20 (500 µL wine / (9,450 µL buffer + 50 µL spike)). This factor is part of the standard curve. Please do not use for calculation.
3.) Use 100 µL directly in the ELISA. Please measure the diluted spiked wine samples within 30 minutes from the time of adding the spike to the wine.

Preparation of a sample with 1.0 ppm Casein (NOT recommended):

1.) Add 20 µL of the 500 ppm Spike Solution (undiluted 5 ppm standard) to 500 µL of wine and mixed thoroughly.
2.) Now add 9,480 µL of pre-diluted sample buffer to the spiked wine and mix again. The dilution factor is 1:20 (500 µL wine / (9,480 µL buffer + 20 µL spike)). This factor is part of the standard curve. Please do not use for calculation.
3.) Use 100 µL directly in the ELISA. Please measure the diluted spiked wine samples within 30 minutes from the time of adding the spike to the wine.

3. Calculation of the results

Actually there is no MRL (maximum residue level) set for casein in all food (wine included). Therefore there is no need to use the recovery rate for calculation. But if you would like to use, then please analyse the same sample – one time unspiked and one time spiked, and calculate as follows:

$$\text{Measured ppm of the sample} \times \frac{100\ \%}{\text{Recovery rate (\%)}} = \text{real casein content in wine}$$

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