

## AlerTox® ELISA Kits Detect Allergenic Wine Fining Agents

A Summary of a BIPEA Proficiency Testing Study for Casein, Lysozyme and Ovalbumin

### What Testing is Needed for Wine Fining Agents

Winemakers routinely add certain proteins (called “finings”) to wines to remove cloudiness, reduce tannins and eliminate off-flavors. Many of these proteins, such as casein, lysozyme and ovalbumin, are allergenic and must be removed before making the wines available for purchase.

Current processes involve chemical precipitation of the added protein. The resulting precipitants settle on the bottom of the vessel for easy filtering. But some regulations, such as in the European Union, require a demonstration that there are no allergens present in the final product, and starting in December 2023, certain [labeling rules](#) would require manufacturers to declare the presence of these allergens, which could significantly affect product sales even if the allergen were no longer present.

The International Organization for Vine and Wine (OIV) has determined that the methods used to detect residues of these allergenic proteins must be able to detect as little as 0.5 ppm (0.5 mg/L) of each protein.

### Description of the AlerTox® ELISA Kits for Wine Fining Agents

AlerTox ELISA Kits are immunosorbent assays designed for the quantitative determination of allergens in raw materials and final products. The kits are based on the ELISA sandwich technique that is often used to analyze substances found at very low concentrations. This method, combined with the high specificity and sensitivity of the antibody used in these tests, allows the kits to precisely quantify allergens in all types of food and drinks.

Wine fining agents include milk and egg proteins that can be detected by using three AlerTox ELISA Kits (Table 1). Samples tested for casein must be extracted separately from the samples used to test for lysozyme and ovalbumin. However, the same wine sample extraction can be used with lysozyme and ovalbumin kits.

**Table 1. AlerTox ELISA Kits that are Ideal for Allergen Testing of Wine Fining Agents.**

Kit	Limit of Detection (ppm)	Limit of Quantification (ppm)
AlerTox ELISA Casein Kit	0.05	0.20
AlerTox ELISA Lysozyme Kit	0.002	0.025
AlerTox ELISA Ovalbumin Kit	0.005	0.025

### Proficiency Testing Method

Proficiency testing (PT) is a way to compare the performance of detection kits on equal terms. PT rounds are organized by independent organizations (e.g., BIPEA\*). They send out the same samples to all participants, collect their results, analyze the data and produce reports that are made public. In these reports, samples are assigned a consensus allergen level (in ppm). In this way, participants can compare how well their kits have performed in detecting the correct amount of allergen. Results are not retractable.

Hygiena participated in BIPEA PT rounds with spiked white wine samples (PT program: 71 Allergens in Wines, Feb 2019). AlerTox ELISA Kit results were submitted after testing for casein, lysozyme and ovalbumin without knowing the protein levels in the samples.

\* BIPEA is a European non-profit organization that offers more than 220 regular proficiency testing programs and includes more than 2600 member laboratories in 130 countries. Their proficiency testing programs cover the fields of grains, milling, agri-food, contaminant(s), environments, cosmetics and pharmaceuticals.



## Results

The BIPEA report (ILCR n° 2018-2019 – 0510) included a comparison to accepted ranges of values. The results are classified as “satisfactory” or “not satisfactory” based on statistical analysis and the creation of a z-score. The z-score is calculated for each result and scored as “satisfactory” if the value is between –2 and +2. As seen in Table 2, the three tests were well within the acceptable values. The casein sample, after being tested with the AlerTox ELISA Casein Kit, gave a result below the test’s limit of quantification (LOQ). The consensus was “Not detected”.

**Table 2. Summary of Results from AlerTox ELISA Kits.**

Sample	Consensus Value*	Hygiena’s AlerTox ELISA Kit Results	Z-score	Conclusion
Casein	Not detected	<LOQ <sup>†</sup>	N/A	Satisfactory
Lysozyme	0.54 ppm	0.49 ppm	–0.29	Satisfactory
Ovalbumin	0.21 ppm	0.11 ppm	–1.33	Satisfactory

\* Consensus value = based on all the results submitted for the analyte being tested using any kit or method.

† LOQ = limit of quantification.

## Conclusions

The BIPEA report shows that the three Hygiena kits work well in detecting the possible presence of the allergenic proteins (casein, lysozyme and ovalbumin) in white wines.

The AlerTox ELISA Ovalbumin and AlerTox ELISA Lysozyme Kits are highly sensitive kits for the detection of ovalbumin and lysozyme quantification residues in wine. AlerTox ELISA Casein is a highly sensitive kit for the detection of casein.

ELISA, PCR and mass spectrometry (MS) are commonly used techniques for allergen detection. However, ELISA is the fastest and least expensive of the techniques and requires the least amount of technical expertise. The BIPEA testing shows that the level of detection from ELISAs is acceptable to regulatory limits and limits of quantification. Furthermore, the three Hygiena kits are OIV-conforming since their quantification limits are below 0.5 ppm.