

AlerTox[®] ELISA

Specific for wine

Express Protocol Of Use

1. Reagents preparation

- 1. Set all reagents at room temperature (20-25°C).**
- 2. Washing Solution:** dilute the solution 1:10 with distilled water. If precipitates are observed in the solution, heat at 37° C for 15 minutes.
- 3. Extraction Solution:** dilute the solution with distilled water using the dilution factors below:

AlerTox Ovalbumin: 1:10

AlerTox Lysozyme: 1:10

AlerTox Casein*: 1:5

***Standards:** dilute all the standards (including 0 ppm standard) 1:100 in diluted Extraction Solution. To obtain a final volume of 2 mL, add 20 µL of concentrated standard and 1,980 µL of diluted Extraction Solution.

2. Sample preparation

- 1.** Add 0.5 mL of sample in a test tube.
- 2.** Add 9.5 mL of diluted Extraction Solution. Close the tube and mix to homogenize
- 3.** Generally, liquid samples do not require a final step of centrifugation, since the mixture is usually clear. If not, centrifuge the sample for 10 minutes at 2,000 x g.

- | | | |
|-------------------|---|---|
| Pipette | → | 100 µL Standard or Sample Extract in duplicates into wells. |
| ↓ | | |
| Incubate | → | at room temperature for 20 minutes. |
| ↓ | | |
| Wash wells | → | 3 times with 300 µL of diluted Washing Solution. |
| ↓ | | |
| Pipette | → | 100 µL of conjugate (anti-antigen-peroxidase) into wells. |
| ↓ | | |
| Incubate | → | at room temperature for 20 minutes. |
| ↓ | | |
| Wash wells | → | 5 times with 300 µL of diluted Washing Solution. |
| ↓ | | |
| Pipette | → | 100 µL of Substrate Solution into wells. |
| ↓ | | |
| Incubate | → | at room temperature for 20 minutes in the dark. |
| ↓ | | |
| Pipette | → | 100 µL of Stop Solution into wells. |
| ↓ | | |
| Mix well and wait | → | 1 minute at room temperature. |
| ↓ | | |
| Read | → | the plate at 450 nm wavelength. |