

AlerTox[®] ELISA

Allergen

ELISA test for the determination of allergens
in food and drinks.

AlerTox[®] ELISA

Allergen

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1. Introduction

This instruction for use is valid for all AlerTox ELISA of the Allergen series. The 20 AlerTox ELISA listed below have an identical protocol, which allows you to determine the content of different allergens in parallel by following the same scheme for sample preparation and the ELISA protocol.

Please do not modify the protocol in respect of the timings, the pipetting volumes, the type of buffers, the pH value of the buffers and the temperature. Any modification of the protocol as described before, will cancel the validation of the test system.

A pH adjustment is generally not necessary. Do not shake the plate during incubation. Do not use kit components after the expiration date.

You can use this instruction for use for the following ELISA kits:

| Item (96 wells) | Reference | |
|--|-------------------|--------------|
| | Manufacturer Code | Hygiene Code |
| AlerTox ELISA Coconut | KT-6285 | KIT3056 |
| AlerTox ELISA Lupine | KT-5914 | KIT3057 |
| AlerTox ELISA Sesame | KT-5908 | KIT3051 |
| AlerTox ELISA Mustard (white, black, brown) | KT-5915 | KIT3058 |
| AlerTox ELISA Macadamia | KT-6287 | KIT3055 |
| AlerTox ELISA Cashew | KT-5916 | KIT3053 |
| AlerTox ELISA Soy (STI = Soy Trypsin Inhibitor) | KT-5906 | KIT3047 |
| AlerTox ELISA Walnut | KT-5909 | KIT3052 |
| AlerTox ELISA Peanut | KT-5905 | KIT3048 |
| AlerTox ELISA Hazelnut | KT-5907 | KIT3050 |
| AlerTox ELISA Almond | KT-5910 | KIT3049 |
| AlerTox ELISA Pistachio | KT-5917 | KIT3054 |
| AlerTox ELISA Egg (Egg white proteins) | KT-5904 | KIT3046 |
| AlerTox ELISA Lysozyme | KT-5757 | KIT3044 |
| AlerTox ELISA Ovalbumin | KT-5759 | KIT3045 |
| AlerTox ELISA Casein | KT-5761 | KIT3043 |
| AlerTox ELISA Milk (Casein & BLG) | KT-5918 | KIT3041 |
| AlerTox ELISA BLG | KT-5919 | KIT3042 |
| AlerTox ELISA Crustacean | KT-5903 | KIT3059 |
| AlerTox ELISA Fish | KT-5920 | KIT3060 |

Sample extracts, prepared with one of the following AlerTox ELISA, can also be used directly in each of them: AlerTox ELISA Coconut, AlerTox ELISA Lupine, AlerTox ELISA Sesame, AlerTox ELISA Mustard, AlerTox ELISA Macadamia, AlerTox ELISA Cashew, AlerTox ELISA Soy (STI), AlerTox ELISA Walnut, AlerTox ELISA Peanut, AlerTox ELISA Hazelnut, AlerTox ELISA Almond, AlerTox ELISA Pistachio, AlerTox ELISA Egg, AlerTox ELISA Lysozyme (only the wine extract is compatible), AlerTox ELISA Ovalbumin and AlerTox ELISA BLG.

That means that you can determine the content of 16 different allergens using one single sample extract.

Samples for AlerTox ELISA Casein, AlerTox ELISA Milk, AlerTox ELISA Crustacean and AlerTox ELISA Fish have to be extracted individually.

2. Limits

| Kit | LOD | LOQ | Kit | LOD | LOQ |
|-------------------------|---------|--------|--------------------------|----------|----------|
| AlerTox ELISA Coconut | 0.4 ppm | 2 ppm | AlerTox ELISA Almond | 0.2 ppm | 0.5 ppm |
| AlerTox ELISA Lupine | 0.2 ppm | 2 ppm | AlerTox ELISA Pistachio | 0.13 ppm | 1 ppm |
| AlerTox ELISA Sesame | 0.2 ppm | 2 ppm | AlerTox ELISA Egg | 0.05 ppm | 0.4 ppm |
| AlerTox ELISA Mustard | 1.5 ppm | 2 ppm | AlerTox ELISA Lysozyme | 2 ppb | 25 ppb |
| AlerTox ELISA Macadamia | 0.1 ppm | 1 ppm | AlerTox ELISA Ovalbumin | 5 ppb | 25 ppb |
| AlerTox ELISA Cashew | 0.2 ppm | 2 ppm | AlerTox ELISA Casein | 0.05 ppm | 0.20 ppm |
| AlerTox ELISA Soy (STI) | 16 ppb | 50 ppb | AlerTox ELISA Milk | 0.05 ppm | 0.5 ppm |
| AlerTox ELISA Walnut | 0.6 ppm | 2 ppm | AlerTox ELISA BLG | 1.5 ppb | 10 ppb |
| AlerTox ELISA Peanut | 0.3 ppm | 1 ppm | AlerTox ELISA Crustacean | 1 ppb | 20 ppb |
| AlerTox ELISA Hazelnut | 0.3 ppm | 1 ppm | AlerTox ELISA Fish | 1.4 ppm | 4 ppm |

3. Quantification ranges

| Kit | Range | |
|--------------------------|--|------------------|
| AlerTox ELISA Coconut | (25-12.5-5-2-0 ppm) | ready-to-use |
| AlerTox ELISA Lupine | (30-15-5-2-0 ppm) | ready-to-use |
| AlerTox ELISA Sesame | (20-10-4-2-0 ppm) | ready-to-use |
| AlerTox ELISA Mustard | (50-25-10-2-0 ppm) | ready-to-use |
| AlerTox ELISA Macadamia | (25-12.5-5-1-0 ppm) | ready-to-use |
| AlerTox ELISA Cashew | (50-25-10-2-0 ppm) | ready-to-use |
| AlerTox ELISA Soy (STI) | (600-300-150-50-0 ppb) | ready-to-use |
| AlerTox ELISA Walnut | (50-15-5-2-0 ppm) | ready-to-use |
| AlerTox ELISA Peanut | (30-15-5-1-0 ppm) | ready-to-use |
| AlerTox ELISA Hazelnut | (40-15-5-1-0 ppm) | ready-to-use |
| AlerTox ELISA Almond | (10-5-2-0.5-0 ppm) | ready-to-use |
| AlerTox ELISA Pistachio | (25-12.5-5-1-0 ppm) | ready-to-use |
| AlerTox ELISA Egg | (8-4-2-0.4-0 ppm) | ready-to-use |
| AlerTox ELISA Lysozyme | (250-125-50-25-0 ppb) | ready-to-use |
| AlerTox ELISA Ovalbumin | (500-250-100-25-0 ppb) | ready-to-use |
| AlerTox ELISA Casein | (5-2.5-1-0.2-0 ppm) (after dilution of the standards) | 100x concentrate |
| AlerTox ELISA Milk | (10-5-2-0.5-0 ppm) (after dilution of the standards) | 100x concentrate |
| AlerTox ELISA BLG | (300-150-50-10-0 ppb) | ready-to-use |
| AlerTox ELISA Crustacean | (400-200-80-20-0 ppb) | ready-to-use |
| AlerTox ELISA Fish | (100-50-20-4-0 ppm) | ready-to-use |

4. Recovery (Tested in typical matrices) O. juice = Orange juice

| | | | | | |
|--------------------------|------------------------------------|------------------------------|------------------|-----------------|--------------------|
| AlerTox ELISA Coconut | Cookies: 92% | Cornflakes: 102% | Ice cream: 74% | Chocolate: 87% | Sausage: 80% |
| AlerTox ELISA Lupine | Sausage: 99% | Biscuit: 113% | O. juice: 104% | Ketchup: 98% | Croquette: 111% |
| AlerTox ELISA Sesame | Sausage: 92% | Cracker: 109% | Inst.-soup: 110% | Dressing: 93% | --- |
| AlerTox ELISA Mustard | Sausage: 98% | Can.-soup: 96% | Inst.-soup: 80% | Dressing: 76% | Herb-mix: 78% |
| AlerTox ELISA Macadamia | Cookies: 106% | Cornflakes: 104% | Ice cream: 92% | Chocolate: 83% | Sausage: 101% |
| AlerTox ELISA Cashew | Cookies: 109% | Cornflakes: 98% | Ice cream: 93% | Chocolate: 102% | --- |
| AlerTox ELISA Soy (STI) | Cookies: 106% Inst.-soup: 90% | Cornflakes: 100% | Ice cream: 77% | Chocolate: 77% | Sausage: 96% |
| AlerTox ELISA Walnut | Cookies: 103% | Cornflakes: 106% | Ice cream: 87% | Chocolate: 60% | --- |
| AlerTox ELISA Peanut | Cookies: 101% | Cornflakes: 100% | Ice cream: 89% | Chocolate: 110% | --- |
| AlerTox ELISA Hazelnut | Cookies: 99% | Cornflakes: 101% | Ice cream: 90% | Chocolate: 83% | --- |
| AlerTox ELISA Almond | Cookies: 90% | Cereals: 105% | Ice cream: 77% | Chocolate: 68% | --- |
| AlerTox ELISA Pistachio | Cookies: 94% | Cornflakes: 96% | Ice cream: 89% | Chocolate: 81% | Sausage: 90% |
| AlerTox ELISA Egg | Cookies: 85% | Pasta: 91% | Biscuit: 83% | Chocolate: 82% | Sausage: 98% |
| AlerTox ELISA Ovalbumin | Red wine: 93% | Rosé wine: 102% | White wine: 100% | --- | --- |
| AlerTox ELISA Lysozyme | Red wine: 99% | Rosé wine: 91% | Cheese: 93% | --- | --- |
| AlerTox ELISA Casein | Red wine: 89% Cookies: 100% | Rosé wine: 80% Bread: 80% | White wine: 102% | Chocolate: 86% | Sausage: 80% |
| AlerTox ELISA Milk | White wine: 122% | Bread: 110% | Sausage: 88% | Chocolate*: 99% | *) on soy base |
| AlerTox ELISA BLG | Cookies: 88% | Cornflakes: 94% | Sausage: 107% | Chocolate: 86% | White wine: 82% |
| AlerTox ELISA Crustacean | Cracker: 90% | Soy sauce: 90% | Fish: 93% | Meat: 97% | --- |
| AlerTox ELISA Fish | Red wine: 103% Spring Roll: 93% | Soup: 117% Surimi: 114% | Asia Sauce: 103% | Cracker: 99% | Worcester S.: 112% |

5. Cross-reactivity (s=seeds; l=leaves; r=roots; n=native; h=heated; sm=smoked; m=meat; gr=granulate; k=kernel; p=powder)

| | | | | |
|-------------------------|---|---|--|---|
| AlerTox ELISA Coconut | Coconut: 100% | --- | --- | --- |
| AlerTox ELISA Lupine | Lupine: 100% Lentil: 0.0004% | Soy flour, n*: 0.07% Chickpea: 0.0003% | Soy lecithin: 0.002% Beef meat: 0.0003% | Soy flour, h: 0.0009% not used in food |
| AlerTox ELISA Sesame | Sesame: 100% | Oat: 0.0003% | Bean: 0.0003% | Chia: 0.36% |
| AlerTox ELISA Mustard | White mustard: 100% | Black mustard: 50% Field mustard: 48% | Brown mustard: 59% | Rape s.: 59% |
| AlerTox ELISA Macadamia | Macadamia: 100% | Walnut: 0.001% | Hazelnut: 0.0002% | --- |
| AlerTox ELISA Cashew | Cashew: 100% | Pistachio: 4% | --- | --- |
| AlerTox ELISA Soy (STI) | STI: 100% | --- | --- | --- |
| AlerTox ELISA Walnut | Walnut: 100% | Pecan nut: 0.0005% | Cashew: 0.0002% | Chicken: 0.0002% |
| AlerTox ELISA Peanut | Peanut: 100% | Gum arabic: 0.003% | --- | --- |
| AlerTox ELISA Hazelnut | Hazelnut: 100% | Walnut: 0.0022% | --- | --- |
| AlerTox ELISA Almond | Almond: 100% Mahaleb k: 1.42% | Peach k: 15.95% Apricot k: 100% | Plum k: 1% | Sweet cherry k: 1.74% |
| AlerTox ELISA Pistachio | Pistachio: 100% Pecan nut: 0.0005% | Cashew: 12% Sunflowers: 0.0002% | Hazelnut: 0.17% --- | Walnut: 0.0008% --- |
| AlerTox ELISA Egg | Egg: 100% | Chicken meat: 0.001% | Ovomucoid: 614% | Conalbumin: 2.60% Ovalbumin: 0.25% |
| AlerTox ELISA Ovalbumin | Ovalbumin: 100% Egg white protein (total): 75% | Conalbumin: <0.2% | Lysozyme: <0.02% | Ovomucoid: <0.02% |
| AlerTox ELISA Lysozyme | Lysozyme: 100% | Ovalbumin: <0.0001% | Conalbumin: <0.0001% | Egg white protein (total): 2.2% |
| AlerTox ELISA Casein | Casein: 100% | Sheep's milk: <1.2% | Goat's milk: <1.1% | --- |

| | | | | |
|--------------------------|--|--|--|--|
| AlerTox ELISA Milk | Caseinat: 100% | BLG: 89.9% | Sheep's milk: 0.96% | Goat's milk: 0.014% |
| AlerTox ELISA BLG | BLG: 100% | Sheep's milk: <0.2% | Casein: <0.02% | Goat's milk: <0.002% |
| AlerTox ELISA Crustacean | Tropomyosin (from <i>Penaeus indicus</i>): 100% | --- | --- | --- |
| AlerTox ELISA Fish | Cod: 100% Carp: 79.6% Coalfish: 31.0% Samlet: 19.6% Spinned loach: 6.3% Salmon: 2.6% Tuna: 0.8% Chicken m.: 0.00035% Buckwheat: 0.00003% | Catfish: 168.0% Red mullet: 58.9% Zander: 30.2% Trout: 18.6% Eel: 5.7% Turbot: 2.5% Mackerel sm.: 0.4% Shrimp: 0.00006% Oat: 0.0003% | Perch: 93.1% Haddock: 53.4% Pangasius: 29.5% Flounder: 13.5% Redfish: 2.8% Herring sm.: 1.5% Swordfish: 0.2% Beef m.: 0.00005% Mustard: 0.00001% | Pike: 85.3% Bass: 46.9% Plaice: 28.9% Sardine: 7.0% Sole: 2.8% Devilfish: 1.1% Sheep m.: 0.0003% Macadamia: 0.00004% Onion: 0.00001% |

The overview about non-cross-reacting matrices, which have been tested per kit, you will find on the next page.

6. Shelf life From date of production

| | | | |
|-------------------------|-----------|--------------------------|-----------|
| AlerTox ELISA Lupine | 13 months | AlerTox ELISA Pistachio | 13 months |
| AlerTox ELISA Sesame | 18 months | AlerTox ELISA Egg | 24 months |
| AlerTox ELISA Mustard | 24 months | AlerTox ELISA Ovalbumin | 24 months |
| AlerTox ELISA Cashew | 13 months | AlerTox ELISA Lysozyme | 24 months |
| AlerTox ELISA Soy (STI) | 24 months | AlerTox ELISA Casein | 13 months |
| AlerTox ELISA Walnut | 13 months | AlerTox ELISA Milk | 13 months |
| AlerTox ELISA Peanut | 13 months | AlerTox ELISA BLG | 13 months |
| AlerTox ELISA Hazelnut | 18 months | AlerTox ELISA Crustacean | 24 months |
| AlerTox ELISA Almond | 24 months | AlerTox ELISA Fish | 24 months |
| AlerTox ELISA Coconut | 13 months | AlerTox ELISA Macadamia | 13 months |

7. Reference list of all tested matrices

| | | | |
|--------------------------|---------------------|-------------------------|--------------------------|
| [01] Almond | [13] Brazil nut | [25] Catfish | [37] Conalbumin |
| [02] Apple fruit flesh | [14] BSA | [26] Cayenne | [38] Corn |
| [03] Apricot fruit flesh | [15] Buckwheat | [27] Celery | [39] Cow's milk |
| [04] Barley | [16] Cabbage | [28] Cherry fruit flesh | [40] Cumin seeds |
| [05] Bass | [17] Cacao | [29] Chestnut | [41] Curcuma |
| [06] Bean | [18] Caraway seeds | [30] Chicken meat | [42] Devilfish |
| [07] Bean, green | [19] Cardamom seeds | [31] Chickpea | [43] Dill |
| [08] Bean, red | [20] Carob gum | [32] Cinnamon | [44] Eel |
| [09] Bean, white | [21] Carp | [33] Clove | [45] Egg |
| [10] Beef gelatine | [22] Carrot | [34] Coalfish | [46] Flounder |
| [11] Beef meat | [23] Casein | [35] Coconut | [47] Garden cress leaves |
| [12] BLG | [24] Cashew nut | [36] Cod | [48] Gliadin |

| | | | |
|---------------------------|------------------------|----------------------------|-----------------------------|
| [49] Goat's milk | [73] Oyster | [97] Rye | [121] Walnut |
| [50] Guar gum | [74] Pangasius | [98] Salmon | [122] Wheat |
| [51] Haddock | [75] Pea | [99] Samlet | [123] Whole milk powder |
| [52] Hazelnut | [76] Peach fruit flesh | [100] Sardine | [124] Wine |
| [53] Herring (smoked) | [77] Peanut | [101] Sesame | [125] Wine, red |
| [54] Horseradish roots | [78] Pecan | [102] Sheep's milk | [126] Wine, rosé |
| [55] Kiwi | [79] Pepper | [103] Shrimp, cooked | [127] Wine, white |
| [56] Leek | [80] Perch | [104] Shrimp, raw | [128] Zander |
| [57] Lentil | [81] Pike | [105] Skim milk powder | [129] Mahaleb cherry kernel |
| [58] Locust bean gum | [82] Pine seeds | [106] Sole | [130] Sweet cherry kernel |
| [59] Lupine | [83] Pistachio | [107] Soy flour, roasted | [131] Peach kernel |
| [60] Lysozyme | [84] Plaice | [108] Soy flour, unroasted | [132] Plum kernel |
| [61] Macadamia | [85] Plum fruit flesh | [109] Soy lecithin | [133] Fenugreek seeds |
| [62] Mackerel (smoked) | [86] Poppy seeds | [110] Spined loach | [134] Chili powder, sweet |
| [63] Millet | [87] Pork gelatine | [111] Strawberry | [135] Paprika powder, sweet |
| [64] Mustard, black seeds | [88] Pork meat | [112] Sucrose | [136] Tofu |
| [65] Mustard, brown seeds | [89] Potato, raw | [113] Sunflower seeds | [137] Chervil |
| [66] Mustard, white seeds | [90] Potato, cooked | [114] Swordfish | [138] Fish gelatine |
| [67] Nutmeg | [91] Pumpkin seeds | [115] Thyme | [139] Isinglass |
| [68] Oats | [92] Radish | [116] Tomato | [140] Egg white powder |
| [69] Onion | [93] Rape seeds | [117] Tropomyosin | [141] Cress, leaves |
| [70] Orange | [94] Red mullet | [118] Trout | |
| [71] Ovalbumin | [95] Redfish | [119] Tuna | |
| [72] Ovomucoid | [96] Rice | [120] Turbot | |

8. Tested non-cross-reactive matrices per kit

| | |
|-------------------------|--|
| AlerTox ELISA Coconut | [01, 77, 52, 121, 78, 13, 61, 29, 82, 24, 83, 113, 91, 85, 28, 03, 76, 55, 122, 04, 68, 97, 38, 96, 107, 109, 136, 15, 22, 27, 137, 31, 57, 59, 09, 66, 75, 101, 86, 116, 89, 141, 50, 20, 139, 103, 104, 30, 88, 11, 17, 48, 112, 36, 39, 102, 49, 45, 140, 10] |
| AlerTox ELISA Lupine | [01, 04, 13, 121, 83, 29, 61, 24, 52, 35, 78, 77, 91, 113, 75, 06, 97, 38, 68, 96, 122, 56, 101, 15, 76, 28, 85, 70, 111, 116, 90, 17, 39, 45, 88, 30, 58, 50] |
| AlerTox ELISA Sesame | [122, 04, 97, 39, 45, 17, 96, 38, 15, 107, 113, 91, 82, 86, 52, 77, 24, 61, 83, 29, 01, 78, 13, 35, 121, 31, 109, 75, 59, 116, 55] |
| AlerTox ELISA Mustard | [16, 79, 41, 26, 33, 67, 32, 43, 115, 75, 06, 97, 38, 04, 68, 122, 96, 107, 101, 15, 22, 56, 27, 36, 39, 45, 11, 88, 30, 92] |
| AlerTox ELISA Macadamia | [01, 77, 78, 13, 61, 29, 82, 24, 83, 113, 91, 85, 28, 03, 76, 55, 122, 04, 68, 97, 38, 96, 107, 109, 136, 15, 22, 27, 137, 31, 57, 59, 09, 66, 75, 101, 86, 116, 89, 141, 50, 20, 139, 103, 104, 30, 88, 11, 17, 48, 112, 36, 39, 102, 49, 45, 140, 10] |

| | |
|--------------------------|---|
| AlerTox ELISA Cashew | [64, 65, 66, 03, 85, 01, 77, 52, 121, 78, 13, 61, 35, 29, 82, 113, 91, 57, 59, 75, 09, 97, 38, 04, 68, 122, 96, 107, 109, 101, 15, 22, 56, 27, 36, 39, 102, 49, 45, 11, 88, 10, 30, 90, 31, 50, 20, 86, 103, 104, 116, 55, 17, 48, 112] |
| AlerTox ELISA Soy (STI) | [122, 04, 97, 68, 38, 77, 52, 01, 45, 17, 112, 10, 88, 11, 30, 96, 75, 31, 06, 39] |
| AlerTox ELISA Walnut | [76, 85, 03, 28, 01, 31, 91, 24, 77, 35, 61, 113, 86, 39, 102, 97, 38, 04, 68, 122, 96, 109, 30, 11, 88, 10, 15, 17, 112, 45] |
| Alertox ELISA Peanut | [75, 31, 06, 91, 121, 83, 13, 35, 78, 48, 01, 113, 86, 97, 38, 04, 68, 96, 122, 107, 109, 101, 15, 17, 123, 02, 10, 40, 59, 135, 134, 129, 130, 131, 132, 133] |
| AlerTox ELISA Hazelnut | [75, 31, 06, 91, 121, 83, 13, 82, 29, 61, 24, 77, 35, 10, 48, 113, 86, 97, 38, 04, 68, 96, 122, 107, 109, 101, 15, 17, 123, 02, 01] |
| AlerTox Almond | [76, 85, 03, 28, 121, 83, 13, 82, 29, 62, 24, 77, 78, 35, 113, 86, 39, 45, 97, 38, 04, 68, 96, 122, 107, 109, 101, 15, 17, 40, 129, 133, 135] |
| AlerTox ELISA Pistachio | [01, 13, 82, 29, 77, 35, 28, 03, 85, 06, 75, 61, 86, 39, 45, 97, 38, 04, 68, 96, 122, 107, 109, 101, 91, 57, 59, 64, 65, 66, 50, 20, 31, 22, 27, 90, 116, 55, 30, 88, 11, 10, 36, 104, 112, 17] |
| AlerTox ELISA Egg | [68, 122, 04, 97, 39, 102, 17, 96, 38, 15, 107, 113, 101, 82, 86, 52, 77, 24, 61, 83, 29, 78, 13, 35, 121, 109, 76, 85, 03, 28, 88, 11, 112] |
| AlerTox ELISA Ovalbumin | [105, 36, 10] |
| AlerTox ELISA Lysozyme | [105, 36, 10] |
| AlerTox ELISA Casein | [12, 122, 97, 68, 04, 38, 96, 45, 101, 112, 30, 88, 11] |
| AlerTox ELISA Milk | [97, 68, 04, 38, 122, 96, 101, 64, 65, 66, 27, 59, 107, 77, 01, 52, 121, 83, 17, 30, 88, 11, 45, 36] |
| AlerTox ELISA BLG | [14, 11, 88, 30, 45, 122, 68, 97, 04, 96, 38, 15, 107, 86, 101, 113, 91, 82, 24, 77, 52, 121, 78, 13, 35, 01, 83, 61, 29, 31, 75, 06, 109, 112, 17, 70, 124, 76, 03, 28, 85] |
| AlerTox ELISA Crustacean | [36, 73, 39, 45, 11, 88, 30, 121, 83, 13, 82, 29, 52, 24, 77, 01, 78, 61, 35, 91, 113, 122, 97, 38, 04, 68, 96, 101, 15, 107, 109, 75, 06, 90, 22, 27, 56] |
| AlerTox ELISA Fish | [04, 97, 107, 63, 101, 52, 01, 78, 13, 121, 77, 06, 75, 90, 22, 45, 27, 39, 83, 24, 91, 113, 122, 96, 38] |

9. Special hints

| | |
|-------------------------|--|
| AlerTox ELISA Coconut | None |
| AlerTox ELISA Lupine | None |
| AlerTox ELISA Sesame | None |
| AlerTox ELISA Mustard | If you don't want cross-reactions with <i>Brassicaceae</i> spec., use the Mustard sensitive (detects only yellow mustard). |
| AlerTox ELISA Macadamia | None |
| AlerTox ELISA Cashew | None |
| AlerTox ELISA Soy (STI) | Raw soy is not used for food. See conversion factors in Section 11. |
| AlerTox ELISA Walnut | None |
| Alertox ELISA Peanut | None |
| AlerTox ELISA Hazelnut | None |
| AlerTox Almond | None |
| AlerTox ELISA Pistachio | None |
| AlerTox ELISA Egg | None |
| AlerTox ELISA Ovalbumin | Only validated for wine matrix. |
| AlerTox ELISA Lysozyme | Only validated for wine matrix and cheese. |

| | |
|--------------------------|--|
| AlerTox ELISA Casein | Due to high matrix effects dilute meat & sausage samples additionally 1:5 with extraction buffer 1x. |
| AlerTox ELISA Milk | Above 70 °C (158 °F) of sample processing temperature you measure only caseins with this kit. Due to high matrix effects dilute meat & sausage samples additionally 1:5 with extraction buffer 1x. |
| AlerTox ELISA BLG | If you wish to detect hydrolysed BLG in e.g. baby food, then contact the supplier. Due to the BLG is very heat sensitive, the sample material should not be processed above 60 °C (140 °F). Above 70 °C (158 °F) the detection will fail due to the progressive structure change of the protein. |
| AlerTox ELISA Crustacean | The extraction temperature is reduced to 40 °C (104 °F). |
| AlerTox ELISA Fish | Only for fresh and lightly processed food products. |

10. Results are measured as

| | | | |
|-------------------------|-----------------------|--------------------------|---|
| AlerTox ELISA Coconut | Whole fresh nut | AlerTox ELISA Almond | Whole nut |
| AlerTox ELISA Lupine | Whole seed | AlerTox ELISA Pistachio | Whole nut |
| AlerTox ELISA Sesame | Whole seed | AlerTox ELISA Egg | Egg-white proteins |
| AlerTox ELISA Mustard | Whole seed | AlerTox ELISA Lysozyme | Lysozyme |
| AlerTox ELISA Macadamia | Whole nut | AlerTox ELISA Ovalbumin | Ovalbumin |
| AlerTox ELISA Cashew | Whole nut | AlerTox ELISA Casein | Whole Caseins |
| AlerTox ELISA Soy (STI) | Soy trypsin inhibitor | AlerTox ELISA Milk | Whole milk proteins |
| AlerTox ELISA Walnut | Whole nut | AlerTox ELISA BLG | beta-lactoglobulin |
| AlerTox ELISA Peanut | Whole seed | AlerTox ELISA Crustacean | Tropomyosin (from <i>Penaeus indicus</i>) |
| AlerTox ELISA Hazelnut | Whole nut | AlerTox ELISA Fish | Fresh fish meat |

11. Important conversion factors

| | | | |
|-------------------------|---------------------|---|--|
| AlerTox ELISA Coconut | Whole fresh coconut | → | Protein; divide by 30; Coconut flakes → Protein; divide by 16 (literature factor) |
| AlerTox ELISA Lupine | Whole seed | → | Protein; divide by 2.4 (literature factor) |
| AlerTox ELISA Sesame | Whole seed | → | Protein; divide by 5.2 (literature factor) |
| AlerTox ELISA Mustard | Whole seed | → | Protein; divide by 3.3 (literature factor) |
| AlerTox ELISA Macadamia | Whole nut | → | Protein; divide by 12.7 (literature factor) |
| AlerTox ELISA Cashew | Whole nut | → | Protein; divide by 5.7 (literature factor) |
| AlerTox ELISA Soy (STI) | Inhibitor | → | Protein (heated); multiply by 190; Protein → Soybean (heated); multiply by 2.5 |
| | Inhibitor | → | Soybean (heated); multiply by 475 |
| | Inhibitor | → | Protein (not heated); multiply by 17; Protein → Soybean (not heated); multiply by 2.5 |
| AlerTox ELISA Walnut | Whole nut | → | Soybean (not heated); multiply by 42.5 (all soy factors are validated factors) |
| | | → | Raw material → Heated material; multiply by 11.2 |
| AlerTox ELISA Walnut | Whole nut | → | Protein; divide by 6.7 (literature factor) |

| | | | |
|--------------------------|---------------|--------|---|
| AlerTox ELISA Peanut | Whole seed | —————> | Protein; divide by 4.0 (literature factor) |
| AlerTox ELISA Hazelnut | Whole nut | —————> | Protein; divide by 7.6 (literature factor) |
| AlerTox ELISA Almond | Whole nut | —————> | Protein; divide by 4.8 (literature factor) |
| AlerTox ELISA Pistachio | Whole nut | —————> | Protein; divide by 5.7 (literature factor) |
| AlerTox ELISA Egg | --- | | |
| AlerTox ELISA Ovalbumin | --- | | |
| AlerTox ELISA Lysozyme | --- | | |
| AlerTox ELISA Casein | Casein | —————> | Skim milk powder; multiply by 3.6 (validated factor) |
| | Casein | —————> | Whole milk; multiply by 42 (validated factor) |
| AlerTox ELISA Milk | Milk proteins | —————> | Skim milk powder; multiply by 2.7 (validated factor) |
| AlerTox ELISA BLG | BLG | —————> | Skim milk powder; multiply by 60 (validated factor) |
| | BLG | —————> | Whole milk; multiply by 370 (validated factor) |
| AlerTox ELISA Crustacean | Tropomyosin | —————> | Shrimp lyophilised; multiply by 70 (validated factor); others see validation report |
| AlerTox ELISA Fish | --- | | |

12. General precautions

- Read this manual carefully before starting the test.
- The test must be performed by specialized and trained staff.
- Handle the test kit in accordance with good laboratory practices (GLP).
- Do not interchange reagents between kits of different lot numbers.
- Do not use reagents beyond the expiration date of the kit.
- The alteration of a reagent can cause inaccurate results.
- Do not exchange the vial caps.
- Use sterile pipette tips.
- Do not use solutions if they become cloudy or precipitate. The only exception is Washing Buffer 10x which may precipitate and must be completely dissolved by warming up at 37°C (99 °F) for 15 minutes before use.
- Use only distilled water for the dilutions of concentrated buffers.
- Substrate solution is light sensitive. Avoid exposure to direct light.
- Do not allow wells to dry completely.
- Handle any solution with gloves.
- During the sample extraction, avoid cross-contamination.
- Devices such as a blender must be cleaned after each sample preparation.
- All reagents must be rebalanced at room temperature (15 - 25 °C/59 - 77 °F) before use.
- Substrate Solution contains TMB, which is highly toxic if inhaled, ingested, or comes in contact with the skin. Please refer to the SDS.
- If you get in contact with toxic or irritating substances, rinse the affected skin area with plenty of water. Please refer to the SDS.
- Stop Solution contains sulphuric acid, which is corrosive. Please refer to the SDS.
- Avoid incubating on cold work benches.

13. Test principle

All AlerTox ELISA tests work on the principle of a quantitative sandwich ELISA. An antibody directed against the target antigen (protein) is bound on the surface of a microtiter plate. Antigen-containing samples or standards are given into the wells of the microtiter plate. After 20 minutes incubation at room temperature (15 - 25 °C/59 - 77 °F), the wells are washed with diluted washing solution to remove unbound material. A peroxidase-conjugated second antibody directed against the same antigen is given into the wells and after 20 minutes of incubation the plate is washed again. A substrate solution is added and incubated for 20 minutes, resulting in the development of a blue color. The color development is inhibited by the addition of a Stop Solution, and the color turns yellow. The yellow color is measured photometrically at 450 nm. The concentration of the antigen is directly proportional to the color intensity of the test sample.

14. Supplied Materials

| Item | Description | 96 wells |
|------|--|-----------|
| 1 | Breakable strips of 8 wells each coated with antigen-specific antibodies. | 12 strips |
| 2 | 5 AlerTox Standards. Ready to use. | 5 x 3 mL |
| 2a | 5 AlerTox Standards; 100x concentrated (only AlerTox ELISA Casein & AlerTox ELISA Milk). | 5 x 1 mL |
| 3 | Conjugate solution. Ready to use. | 1 x 15 mL |
| 4 | Substrate solution (TMB). Ready to use. | 1 x 15 mL |
| 5 | Stop Solution, containing H ₂ SO ₄ . Ready to use. | 1 x 15 mL |
| 6 | Extraction & Sample Dilution Buffer 10x. | 4 x 30 mL |
| 6a | Extraction & Sample Dilution Buffer 5x (only AlerTox ELISA Casein & AlerTox ELISA Milk). | 4 x 60 mL |
| 7 | Washing Solution 10x. | 2 x 60 mL |

15. Storage advice

- All kit components should be kept at 2 – 8 °C (36 - 46 °F) in the dark. DO NOT FREEZE.
- Return all reagents to 2 – 8 °C (36 - 46 °F) immediately after use.
- The diluted Washing Solution concentrate can be used for 4 weeks, when stored at 2 – 8 °C (36 - 46 °F).
- The diluted Extraction & Sample Dilution Buffer can be used for 1 week, when stored at 2 – 8 °C (36 - 46 °F). When crystals precipitate while storing, warm up to 37 °C (99 °F) for 15 min. before use.
- The Sample Extracts are stable for at least 24 hours at 2 – 8 °C (36 - 46 °F), or frozen for longer storage.

16. Material required but not provided

- Multi-channel pipette 50-200 µL.
- Sterile pipette tips.
- Pipettes 10-100 µL, 100-1000 µL.
- ELISA Plate Reader with filter (450 nm).
- Water bath (adjustable to 60 °C (140 °F)).
- 15-30 ml recipients for the extraction.
- Centrifuge.
- Distilled water.
- Stomacher, Mill, Mortar, Blender, etc.
- Vortex mixer.

17. Optional materials/equipment

- Homogeniser for sample extraction.
- The use of a repeating pipette minimises the assay drift.
- An ELISA plate washer system reduces the washing time and improves consistency.
- Use fully automated ELISA analysers (ELISA robots) for more convenience.

18. Reagents preparation

It is advisable to prepare reagents immediately before use and limited to the amount necessary for the number of samples plus the 5 standards, each in duplicates. Please note that all reagents must be at room temperature (15 – 25 °C/59 - 77 °F) at the time of use.

Preparation of the standards (only AlerTox ELISA Casein and AlerTox ELISA Milk).

Dilute all Standards (incl. the Zero-Standard) 1:100 with diluted extraction buffer (20 µl of standard + 1980 µl of diluted extraction buffer).

Please note: The concentrations shown in the standard-curve are those of the 1:100 diluted standards.

Preparation of the Washing Buffer.

Dilute 1:10 with distilled water; warm-up for 15 minutes at 37 °C (99 °F), if precipitated.

Preparation of the Extraction & Sample Dilution Buffer.

Dilute 1:10 with distilled water. (Only for AlerTox ELISA Casein and AlerTox ELISA Milk:

Dilute 1:5 with distilled water).

FOR SOLID SAMPLES: 0.5 g of sample plus 10 ml of the prepared Extraction & Sample Dilution Buffer has to be used.

FOR LIQUID SAMPLES: 0.5 ml of sample plus 9.5 ml of the prepared Extraction & Sample Dilution Buffer has to be used.

ELISA plate

Cut the foil bag beyond the zip. Take out only the number of strips required for the test to be executed (samples plus the 5 standards, both in duplicates) and put them onto the frame. Wells not required must be kept together with the drying agent in the foil bag, well-sealed, and stored at 2 – 8 °C (36 - 46 °F).

19. Sample preparation

a) Wine samples:

1. Add 0.5 ml of wine to 9.5 ml of extraction buffer 1x and mix.
2. Use directly 100 µl in the ELISA plate. Centrifugation is normally not necessary as long as the mixture is clear. If not, centrifuge as described under b) point 4. For other liquid samples proceed with b) point 3.

b) Other kinds of samples:

1. To maximize homogeneity and representativeness of the sample drawing, a minimum of 5 g sample should be pulverized finely in a mortar, impact mill or a similar device.
2. 0.5 g of the homogenized mixture is suspended in 10 ml of extraction buffer 1x.
3. Incubate the mixture for 15 min in a pre-heated water bath at 60 °C (140 °F) (AlerTox ELISA Crustacean 40 °C (104 °F)). To ensure good homogeneity, the samples should be shaken every two minutes.
4. Centrifuge the mixtures for 10 minutes at 2000 x g. If it is not possible to separate the supernatant from the precipitate completely, then filter the supernatant after centrifugation. Cool down the filtrate to room temperature (15 - 25 °C/59 - 77 °F).

(For AlerTox ELISA Casein and AlerTox ELISA Milk kits see also special hints on point 9. Special Hints).

5. 100 μl of this solution are applied per well. If the results of a sample are out of the measuring range, further dilution with the pre-diluted Extraction and Sample dilution buffer is necessary. The additional dilution must be considered when calculating the concentration.

20. Rinsing protocol (Plate washing is a very important step!)

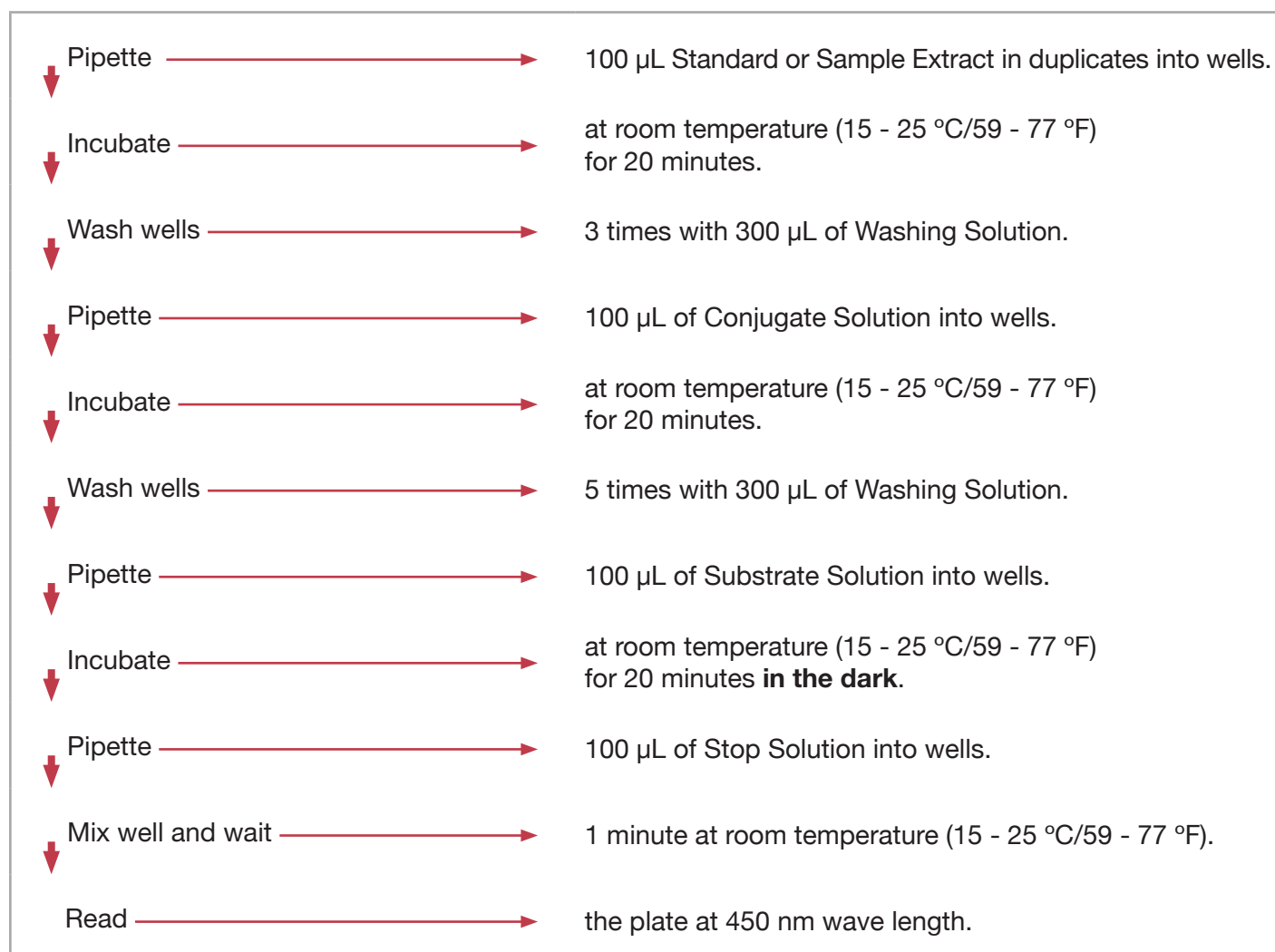
A) Manual rinsing:

Empty the wells. Pipette 300 μl of Washing Buffer into each well. Empty the wells and remove residual liquid by striking the plate against a paper towel. Then repeat washing as advised. The wash procedure is critical. Insufficient washing will result in poor precision and false results.

B) Washer rinsing:

Maintenance of the automated washer is critical. Program the washer to wash with a volume of 300 μl . After the final wash, strike the plate against a paper towel.

21. Scheme of test execution



22. Calculation of the results

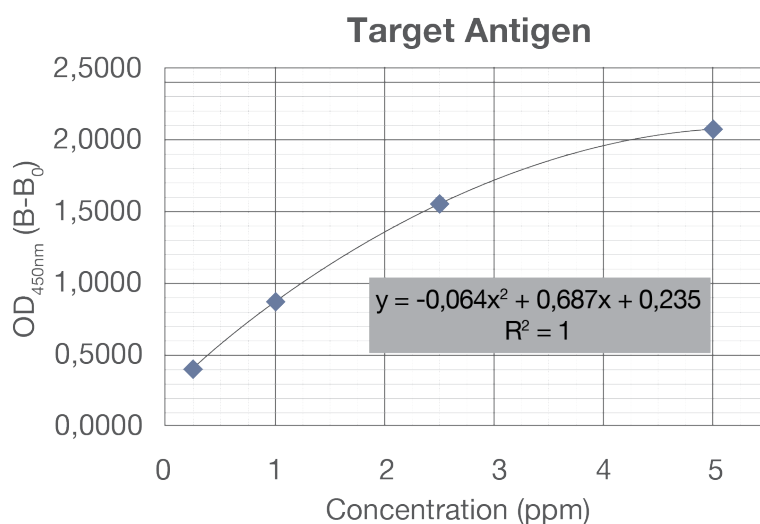
1. Calculate the mean OD_{450nm} value for each set of duplicates of standards and samples.
2. Subtract, from the mean OD_{450nm} value of each standard, the mean OD_{450nm} value of the zero standard.
3. Use the subtracted values of standard 1 to standard 4 to construct the standard curve on the “y”-axis versus the concentration of antigen in ppm or ppb units (depending on the kit) on the “x”-axis.
4. For each diluted sample, find the mean OD_{450nm} value in the “y” axis. Then, using the standard curve, read on the “x” axis the corresponding value for the concentration of antigen.

NOTE!: For the calculation of B-B₀ define the Zero Standard on the reader-software as a Blank. The result will be the same.

Example assay data

| Standard | Target Antigen [ppm] | ODmean _{450nm} | B-B ₀ |
|----------|----------------------|-------------------------|------------------|
| Zero | 0.0 | 0.108 | - |
| 1 | 2.0 | 0.265 | 0.157 |
| 2 | 10.0 | 0.606 | 0.498 |
| 3 | 25.0 | 1.193 | 1.085 |
| 4 | 50.0 | 1.928 | 1.820 |

Example standard curve



Example assay layout

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|-----|-----|------|------|------|------|---|---|---|----|----|----|
| A | S0 | S0 | SP4 | SP4 | SP12 | SP12 | | | | | | |
| B | S1 | S1 | SP5 | SP5 | Etc. | Etc. | | | | | | |
| C | S2 | S2 | SP6 | SP6 | Etc. | Etc. | | | | | | |
| D | S3 | S3 | SP7 | SP7 | Etc. | Etc. | | | | | | |
| E | S4 | S4 | SP8 | SP8 | Etc. | Etc. | | | | | | |
| F | SP1 | SP1 | SP9 | SP9 | Etc. | Etc. | | | | | | |
| G | SP2 | SP2 | SP10 | SP10 | Etc. | Etc. | | | | | | |
| H | SP3 | SP3 | SP11 | SP11 | Etc. | Etc. | | | | | | |

S0: Zero-Standard (without antigen); the mean value = B_0 ; **S1-4:** Standards; the mean value = B;

SP: Samples; the mean value = B.

23. Disclaimer

These products are made from high quality raw materials. No warranty of any kind is made either expressed or implied, as to their suitability other than to measure the target antigen content when used exactly in accordance with these instructions, except regarding the quality of this materials.

Use of the kit for any other purpose is outside its intended use. Any damages, including consequential or special damage or expense arising directly or indirectly from using this product, are limited to the replacement value of the kit.