

AlerTox® ELISA Allergen

ELISA test for the quantitative determination of allergens in food

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AlerTox[®] ELISA Allergen

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1. Introduction

This instruction for use is valid for all Allergen ELISA of the AlerTox series. Actually 18 AlerTox ELISA as being listed below, have an identically protocol, which allows you to work some kits for different parameters parallel by following the same scheme for the 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.

A pH adjustment for is generally not necessary. The buffer capacity of the extraction buffer is doing it for you. Do not shake the plate during incubation. Any modification as described before, will cancel the validation of the test system. Do not use kit components, when being expired.

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

Item	Reference	
	48 wells	96 wells
AlerTox Lupine	KT-6104	KT-5914
AlerTox Sesame	KT-6105	KT-5908
AlerTox Mustard (white, black and brown)	KT-6106	KT-5915
AlerTox Cashew	KT-6107	KT-5916
AlerTox Soy (STI= Soy Trypsin Inhibitor)	KT-6108	KT-5906
AlerTox Walnut	KT-6109	KT-5909
AlerTox Peanut	KT-6110	KT-5905
AlerTox Hazelnut	KT-6111	KT-5907
AlerTox Almond	KT-6112	KT-5910
AlerTox Pistachio	KT-6113	KT-5917
AlerTox Egg (Egg white proteins)	KT-6114	KT-5904
AlerTox Lysozyme	KT-6115	KT-5757
AlerTox Ovalbumin	KT-6116	KT-5759
AlerTox Casein	KT-6117	KT-5761
AlerTox Milk (Casein & BLG)	KT-6118	KT-5918
AlerTox BLG	KT-6119	KT-5919
AlerTox Crustacean	KT-6120	KT-5903
AlerTox Fish	KT-6121	KT-5920

Sample extracts, prepared with one of the following AlerTox ELISA, can also be used directly in each of them: AlerTox Lupine (KT-5914), AlerTox Sesame (KT-5908), AlerTox Mustard (KT-5915), AlerTox Cashew (KT-5916), AlerTox Soy (STI) (KT-5906), AlerTox Walnut (KT-5909), AlerTox Peanut (KT-5905), AlerTox Hazelnut (KT-5907), AlerTox Almond (KT-5910), AlerTox Pistachio (KT-5917), AlerTox Egg (KT-5904), AlerTox Lysozyme (KT-5757), AlerTox Ovalbumin (KT-5759) and AlerTox BLG (KT-5919).

That means that you can work up to 14 parameters out of one single sample extrad.

Samples for AlerTox Casein (KT-5761), AlerTox Milk (KT-5918), AlerTox Crustacean (KT-5903) and AlerTox Fish (KT-5920) have to be extracted individually.

2. Limits

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

3. Quantification ranges

Kit	Range	
AlerTox Lupine	(30-15-5-2-0 ppm)	Dyed rose; ready-to-use
AlerTox Sesame	(20-10-4-2-0 ppm)	Dyed rose; ready-to-use
AlerTox Mustard	(50-25-10-2-0 ppm)	Dyed rose; ready-to-use
AlerTox Cashew	(50-25-10-2-0 ppm)	Dyed rose; ready-to-use
AlerTox Soy (STI)	(600-300-150-50-0 ppb)	Dyed rose; ready-to-use
AlerTox Walnut	(50-15-5-2-0 ppm)	Dyed rose; ready-to-use
AlerTox Peanut	(30-15-5-1-0 ppm)	Dyed rose; ready-to-use
AlerTox Hazelnut	(40-15-5-1-0 ppm)	Dyed rose; ready-to-use
AlerTox Almond	(10-5-2-0.5-0 ppm)	Dyed rose; ready-to-use
AlerTox Pistachio	(25-12.5-5-1-0 ppm)	Dyed rose; ready-to-use
AlerTox Egg	(8-4-2-0.5-0 ppm)	Dyed rose; ready-to-use
AlerTox Lysozyme	(250-125-50-25-0 ppb)	Dyed rose; ready-to-use
AlerTox Ovalbumin	(500-250-100-25-0 ppb)	Dyed rose; ready-to-use
AlerTox Casein	(5-2.5-1-0.20-0 ppm)	Dyed blue; 100X concentrate
AlerTox Milk	(10-5-2-0.5-0 ppm)	Dyed blue; 100X concentrate
AlerTox BLG	(300-150-50-10-0 ppb)	Dyed rose; ready-to-use
AlerTox Crustacean	(400-200-80-20-0 ppb)	Dyed rose; ready-to-use
AlerTox Fish	(100-50-20-4-0 ppb)	Dyed rose; ready-to-use

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

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

5. Cross-reactivity (s=seeds; l=leaves; r=roots; n=naive; h=heated; sm=smoked; m=meat)

AlerTox Lupine	Lupine: 100%	Soybean n*: 0.07%	Soy lecithin: 0.002%	Soybeat h: 0.009%
	Lentil: 0.0004%	Chickpea: 0.0003%	Beef meat: 0.0003%	--- *) not used in food
AlerTox Sesame	Sesame: 100%	Oat: 0.0003%	Bean: 0.0003%	---
AlerTox Mustard	White mustard: 100%	Black mustard: 32.5%	Brown mustard: 26.5%	Rape s.: 15.5%
	Cardamom s.: 0.006%	Caraway s: 0.0012%	Horseradish: 0.0007%	Garden cress l.: 0.0009%
	Cumin s.: 0.0003%	---	---	---
AlerTox Cashew	Cashew: 100%	Pistachio: 4%	---	---
AlerTox Soy (STI)	STI: 100%	Sesame: 0.0002%	---	---
AlerTox Walnut	Walnut: 100%	Pecan nut: 0.85%	Chestnut: 0.11%	Hazelnut: 0.022%
	Pine s.:0.0024%	Sesame: 0.024%	W. mustard: 0.0023%	B. mustard: 0.0023%
	Pistachio: 0.0013%	B. mustard: 0.0009%	Soy: 0.0006%	Brazil nut: 0.0005%
AlerTox Peanut	Peanut: 100%	---	---	---
AlerTox Hazelnut	Hazelnut: 100%	---	---	---
AlerTox Almond	Almond: 100%	---	---	---

AlerTox Pistachio	Pistachio: 100% Pecan nut: 0.005%	Cashew: 12% Sunflowers: 0.0002%	Hazelnut: 0.17% ---	Walnut: 0.0008% ---
AlerTox Egg	Egg: 100%	Chicken meat: 0.001%	---	---
AlerTox Ovalbumin	Ovalbumin: 100%	Conalbumin: <0.2%	Lysozyme: <0.02%	Ovomucoid: <0.02%
AlerTox Lysozyme	Lysozyme: 100%	Ovalbumin: <0.0001%	Conalbumin: <0.0001%	---
AlerTox Casein	Casein: 100%	Sheep's milk: <1.2%	Goat's milk: <1.1%	---
AlerTox Milk	Caseinat: 100%	BLG: 89.9%	Sheep's milk: 0.96%	Goat's milk: 0.014%
AlerTox BLG	BLG: 100%	Sheep's milk: <0.2%	Casein: <0.02%	Goat's milk: <0.002%
AlerTox Crustacean	Tropomyosin: 100%	(of Penaeus indicus)	---	---
AlerTox Fish	Cod: 100%	Catfish: 168.0%	Perch: 93.1%	Pike: 85.3%
	Carp: 79.6%	Red mullet: 58.9%	Haddock: 53.4%	Bass: 46.9%
	Coalfish: 31.0%	Zander: 30.2%	Pangasius: 29.5%	Plaice: 28.9%
	Samlet: 19.6%	Trout: 18.6%	Flounder: 13.5%	Sardine: 7.0%
	Spinned loach: 6.3%	Eel: 5.7%	Redfish: 2.8%	Sole: 2.8%
	Salmon: 2.6%	Turbot: 2.5%	Herring sm.: 1.5%	Devilfish: 1.1%
	Tuna: 0.8%	Mackerel sm.: 0.4%	Swordfish: 0.2%	Sheep m.: 0.0003%
	Chicken m: 0.00035%	Shrimp: 0.00006%	Beef m.: 0.00005%	Macadamia: 0.00004%
	Buckwheat: 0.00003%	Oat: 0.0003	Mustard: 0.00001%	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 (also after opening of the test system!))

AlerTox Lupine	13 months	AlerTox Pistachio	13 months
AlerTox Sesame	15 months	AlerTox Egg	18 months
AlerTox Mustard	15 months	AlerTox Ovalbumin	13 months
AlerTox Cashew	13 months	AlerTox Lysozyme	13 months
AlerTox Soy (STI)	18 months	AlerTox Casein	13 months
AlerTox Walnut	13 months	AlerTox Milk	13 months
AlerTox Peanut	13 months	AlerTox BLG	13 months
AlerTox Hazelnut	18 months	AlerTox Crustacean	13 months
AlerTox Almond	18 months	AlerTox Fish	13 months

7. Reference list of all tested matrices

[01] Almond	[07] Bean, green	[13] Brazil nut	[19] Cardamom seeds
[02] Apple	[08] Bean, red	[14] BSA	[20] Carob gum
[03] Apricot	[09] Bean, white	[15] Buckwheat	[21] Carp
[04] Barley	[10] Beef gelatine	[16] Cabbage	[22] Carrot
[05] Bass	[11] Beef meat	[17] Cacao	[23] Casein
[06] Bean	[12] BLG	[18] Caraway seeds	[24] Cashew nut

[25] Catfish	[51] Haddock	[77] Peanut	[103] Shrimp, cooked
[26] Cayenne	[52] Hazelnut	[78] Pecan	[104] Shrimp, raw
[27] Celery	[53] Herring (smoked)	[79] Pepper	[105] Skim milk powder
[28] Cherry	[54] Horseradish roots	[80] Perch	[106] Sole
[29] Chestnut	[55] Kiwi	[81] Pike	[107] Soy flour, roasted
[30] Chicken meat	[56] Leek	[82] Pine seeds	[108] Soy flour, unroasted
[31] Chickpea	[57] Lentil	[83] Pistachio	[109] Soy lecithin
[32] Cinnamon	[58] Locust bean gum	[84] Plaice	[110] Spined loach
[33] Clove	[59] Lupine	[85] Plum	[111] Strawberry
[34] Coalfish	[60] Lysozyme	[86] Poppy seeds	[112] Sucrose
[35] Coconut	[61] Macadamia	[87] Pork gelatine	[113] Sunflower seeds
[36] Cod	[62] Mackerel (smoked)	[88] Pork meat	[114] Swordfish
[37] Conalbumin	[63] Millet	[89] Potato, raw	[115] Thyme
[38] Corn	[64] Mustard, black seeds	[90] Potato, cooked	[116] Tomato
[39] Cow's milk	[65] Mustard, brown seeds	[91] Pumpkin seeds	[117] Tropomyosin
[40] Cumin seeds	[66] Mustard, white seeds	[92] Radish	[118] Trout
[41] Curcuma	[67] Nutmeg	[93] Rape seeds	[119] Tuna
[42] Devilfish	[68] Oats	[94] Red mullet	[120] Turbot
[43] Dill	[69] Onion	[95] Redfish	[121] Walnut
[44] Eel	[70] Orange	[96] Rice	[122] Wheat
[45] Egg	[71] Ovalbumin	[97] Rye	[123] Whole milk powder
[46] Flounder	[72] Ovomuroid	[98] Salmon	[124] Wine
[47] Garden cress leaves	[73] Oyster	[99] Samlet	[125] Wine, red
[48] Gliadin	[74] Pangasius	[100] Sardine	[126] Wine, rosé
[49] Goat's milk	[75] Pea	[101] Sesame	[127] Wine, white
[50] Guar gum	[76] Peach	[102] Sheep's milk	[128] Zander

8. Tested non-cross-reactive matrices per kit

AlerTox 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 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 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 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 Soy (STI)	[122, 04, 97, 68, 38, 77, 52, 01, 45, 17, 112, 10, 88, 11, 30, 96, 75, 31, 06, 39] [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 Walnut	[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]
AlerTox Peanut	[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]
AlerTox 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 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 Ovalbumin	[105, 36, 10]
AlerTox Lysozyme	[105, 36, 10]
AlerTox Casein	[12, 122, 97, 68, 04, 38, 96, 45, 101, 112, 30, 88, 11]
AlerTox 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 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 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 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 Lupine	None
AlerTox Sesame	None
AlerTox Mustard	If you don't want cross-reactions with Brassicacea spec., use the Mustard sensitive f (detects only yellow mustard).
AlerTox Cashew	None
AlerTox Soy (STI)	Raw soy is not used for food. If you have raw soy in feed, use the conversion factor: [Raw * 11.15 = cooked]. For the detection soy lecithin (always containing soy proteins), use the competitive Soy ELISA.
AlerTox Walnut	None
AlerTox Peanut	None
AlerTox Hazelnut	None
AlerTox Almond	None
AlerTox Pistachio	None
AlerTox Egg	None
AlerTox Ovalbumin	Only validated for wine matrix
AlerTox Lysozyme	Only validated for wine matrix
AlerTox Casein	Due to high matrix effects dilute meat & sausage samples additionally 1:5 with extraction buffer 1X.
AlerTox Milk	Above 70°C 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 BLG	If you wish to detect hydrolysed BLG in e.g. baby food, then use the Non-E-Type BLG kit. Due to the BLG is very heat sensitive, the sample material should not have been processed above 60°C. Above 70°C the detection will fail due to the progressive structure change of the protein.
AlerTox Crustacean	The extraction temperature is reduced to 40°C.
AlerTox Fish	Only for fresh and lightly processed food products.

10. Results are measured as

AlerTox Lupine	Whole seed	AlerTox Pistachio	Whole nut
AlerTox Sesame	Whole seed	AlerTox Egg	Whole egg-white proteins
AlerTox Mustard	Whole seed	AlerTox Ovalbumin	Ovalbumin
AlerTox Cashew	Whole nut	AlerTox Lysozyme	Lysozyme
AlerTox Soy (STI)	Soy trypsin inhibitor	AlerTox Casein	Whole Caseins
AlerTox Walnut	Whole nut	AlerTox Milk	Whole milk proteins
AlerTox Peanut	Whole seed	AlerTox BLG	beta-lactoglobulin
AlerTox Hazelnut	Whole nut	AlerTox Crustacean	Tropomyosin
AlerTox Almond	Whole nut	AlerTox Fish	Fresh fish meat

11. Important conversion factors

AlerTox Lupine	Whole seed	→ Protein; divide by 2.4 (literature factor)
AlerTox Sesame	Whole seed	→ Protein; divide by 5.2 (literature factor)
AlerTox Mustard	Whole seed	→ Protein; divide by 3.3 (literature factor)
AlerTox Cashew	Whole nut	→ Protein; divide by 3.3 (literature factor)
AlerTox Soy (STI)	Inhibitor	→ Protein (heated); multiply with 190 Soybean (heated); multiply with 2.5 (validated factors)
AlerTox Walnut	Whole nut	→ Protein; divide by 6.7 (literature factor)
AlerTox Peanut	Whole seed	→ Protein; divide by 4.0 (literature factor)
AlerTox Hazelnut	Whole nut	→ Protein; divide by 7.6 (literature factor)
AlerTox Almond	Whole nut	→ Protein; divide by 4.8 (literature factor)
AlerTox Pistachio	Whole nut	→ Protein; divide by 5.7 (literature factor)
AlerTox Egg	—	
AlerTox Ovalbumin	—	
AlerTox Lysozyme	—	
AlerTox Casein	Casein	→ Skim milk powder; multiply with 3.6 (validated factor)
AlerTox Milk	Milk proteins	→ Skim milk powder; multiply with 2.7 (validated factor)
AlerTox BLG	BLG	→ Skim milk powder; multiply with 60 (validated factor)
AlerTox Crustacean	Tropomyosin	→ Shrimp lyophilised; multiply with 70 (validated factor); others see validation report
AlerTox Fish	—	

12. General precautions

- Only for in vitro diagnostic use.
- The test must be performed by specialised, trained, and authorised staff.
- Never pipette reagents with the mouth.
- Do not use reagents after the due date indicated on the label.
- 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 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.
- Do not add preservatives or other solutions.
- 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 before use.
- Substrate Solution contains TMB, which is highly toxic if inhaled, ingested, or comes into contact with the skin.
- If you get in contact with toxic or irritating substances, rinse the affected skin area with plenty of water.
- Handle the test kit in accordance with good laboratory practices (GLP).
- Stop Solution contains sulphuric acid, which is corrosive.
- Avoid incubating on cold work benches.

13. Test principle

All Allergen ELISA tests of the AlerTox series are working 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, 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 colour. The colour development is inhibited by the addition of a stop solution, and the colour turns yellow. The yellow colour is measured photometrically at 450 nm.

The concentration of the antigen is directly proportional to the colour intensity of the test sample.

14. Supplied materials

Item	Description	48 wells	96 wells
1	Microtiter strips (8 wells each) coated with antigen-specific antibodies. Ready to use.	6 strips	12 strips
2	5 AlerTox Standards. Ready to use.	5 x 2 mL	5 x 3 mL
o 2a	5 AlerTox Standards; 100X concentrated (only AlerTox Casein & AlerTox Milk)	5 x 1 mL	5 x 1 mL
3	Conjugate solution. Ready to use.	1 x 7,5 mL	1 x 15 mL
4	Substrate solution (TMB). Ready to use.	1 x 7,5 mL	1 x 15 mL
5	Stop Solution, containing H ₂ SO ₄ . Ready to use.	1 x 7,5 mL	1 x 15 mL
6	Extraction & Sample Dilution Buffer 10X.	2 x 30 mL	4 x 30 mL
o 6a	Extraction & Sample Dilution Buffer 5X (only AlerTox Casein & AlerTox Milk)	2 x 60 mL	4 x 60 mL
7	Washing Solution 10x.	1 x 60 mL	2 x 60 mL

15. Storage advice

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

16. Material required but not provided

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

17. Optional materials/equipment

- Homogeniser for Test Portion extraction.
- The use of a repeating pipette minimises the assay drift.
- An ELISA plate washer system reduces the washing time and improves consistency.
- Fully automated ELISA analysers (ELISA robots) for more convenience. Less effort with programming, due to identically protocols. Gives you more time for other tasks.

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 (20-25°C) at the time of use.

Preparation of the standards (only Alertox Casein and Alertox 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 concentration shown in 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, when being precipitated.

Preparation of the extraction & sample dilution buffer.

Dilute 1:10 with distilled water. (Only for Alertox Casein and Alertox 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 along the transverse side beyond the zip. Take out only the number of strips required for the tests to be executed (samples plus the 5 standards, both in duplicates) and put them onto the frame. Wells not required are kept together with the drying agent in the foil bag, well-sealed, and stored at 2–8°C.

19. Sample preparation

Use only the actual manual being supplied with the kit. Do not use older versions!

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. Centrifugation is normally not necessary as long as the mixture is clear. If not, then please centrifuge as being described under b).

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 etc.
2. 0.5 g of the homogenized mixture is suspended in 10 ml of extraction buffer 1X. Afterwards the suspension is incubated for 15 min in a pre-heated water bath at 60°C (Crustacean kit 40°C). To ensure good homogeneity, the samples should be shaken every two minutes.
3. The samples are centrifuged for 10 minutes at 2000xg. 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.(For Casein and Milk kit see also special hints on point 9. Special Hints).
4. 100 µl of particle-free solution are applied per well. If the results of a sample are above of the quantificationrange (do not extrapolate, because you are out of linearity), further dilution with the diluted extraction and sample dilution buffer is necessary. The additional dilution has to be considered when calculating the concentration.

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

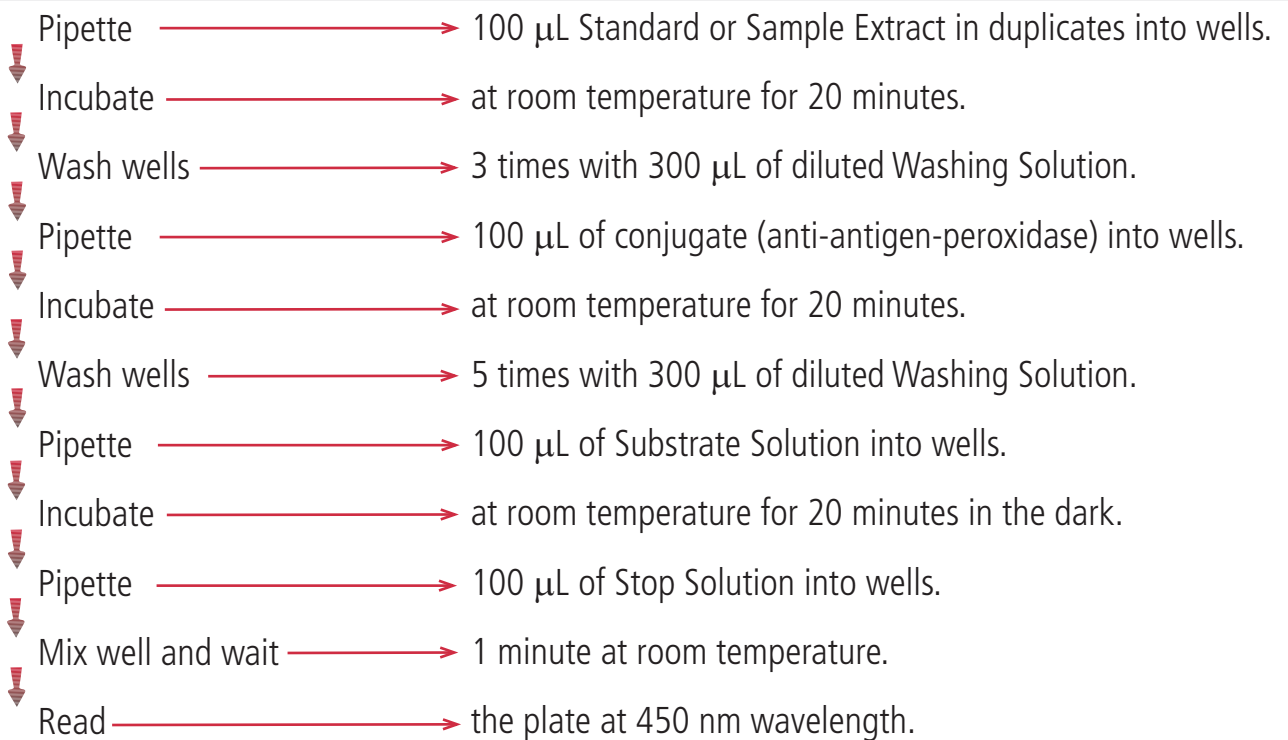
A) Manual rinsing:

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

B) Washer rinsing:

Initial rinsing check: Take an old empty plate with 1 strip. Let the washer fill the wells of the strip with water. All wells should have the same filling level of 300 µl (check!). Let the washer suck out the water of all wells. The wells have to be completely empty and no drops have to be left (check! If not, clean the relevant nozzle and repeat the initial rinsing check). Remove the old plate. Now empty the wells of the test plate manually and place it into the washer or place the filled plate directly into the washer (depends on model). Let the washer wash each well with 300 µl as being advised. Take out and inspect the plate whether all wells are completely empty or not. If not, strike the plate against a paper towel.

Flow scheme of the test execution



21. Calculation of the results

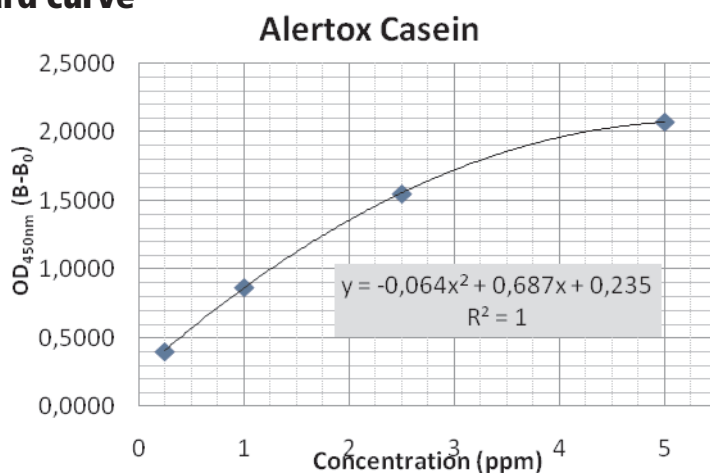
Calculate the mean OD-value (OD_{450nm}) for each set of reference standards or samples.

1. Then subtract from each mean OD-value of all standards and samples the mean value of the zero-standard ($Abs. - Abs._{std. "0"} = B-B_0$).
2. Use the reduced OD-values of standard 1 to standard 4 for your standard curve on the "y"-axis versus the concentration of target-antigen in ppm or ppb (depends on kit) units on the "x"-axis.
3. For each sample-extract find the value B-B₀ on the "y" axis. Then read on the "x" axis the corresponding value for the concentration of the target-antigen. It is not necessary to multiply the resulting concentration of the foodstuff sample by the dilution factor of 20.

Example assay data

Standard	Antigen [ppm]	Mean OD _{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	St0	St0	S4	S4	S12	S12						
B	St1	St1	S5	S5	Etc.	Etc.						
C	St2	St2	S6	S6	Etc.	Etc.						
D	St3	St3	S7	S7	Etc.	Etc.						
E	St4	St4	S8	S8	Etc.	Etc.						
F	S1	S1	S9	S9	Etc.	Etc.						
G	S2	S2	S10	S10	Etc.	Etc.						
H	S3	S3	S11	S11	Etc.	Etc.						

S0: Zero-Standard (without antigen); the mean value = B₀ ; **S1-4:** Standards; the mean value = B ; **SP:** Samples; the mean value = B

22. Disclaimer

This products is 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 casein 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.



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