

Bio-Check (UK)



FISH-CHECK ELISA

A quantitative **FISH ELISA Kit** to assist in the determination of low levels of **FISH (esp. Gadiformes) PROTEIN** in raw materials, environmental swabs, food products.

The prevalence of food allergies is increasing, the age at onset is decreasing and the condition can have severe effects, including breathing difficulties, anaphylaxis and in rare instances, death. Accordingly, new legislation has been put in place to ensure that allergy sufferers are informed of the presence of key food allergens. In the EC, Directives 2003/89 & 2005/26 became fully implemented in November 2005 and in the USA, the Food Allergen Labeling and Consumer Protection Act (FALCPA) came into force early in 2006; both mandate the clear labelling of particular food allergens. In the EC the allergens for which legislation specifically demands inclusion in the ingredient list are: celery/celeriac; cereals (wheat, rye, spelt/kamut or their hybridised strains, barley, oats); crustacea; eggs; fish; lupin; molluscs; mustard; peanuts; sesame; soya & tree nuts (almond, Brazil, cashew, hazelnut, macadamia/Queensland, pecan pistachio, walnut). In the USA the list is similar but excludes celery/celeriac, lupin, mollusc, mustard & sesame. Food producers are being advised by food protection agencies such as the UK's FSA and the US FDA to use best practice by restricting the use of so-called "may contain" labelling to products whose manufacturing processes have been assessed using HACCP and for which the presence of undeclared allergens is both significant and unavoidable.

FISH ALLERGENS in FOOD: Fish, particularly "white" fish like cod, haddock and whiting are relatively common food components, however a significant number of food allergic individuals can suffer mild to severe reactions to fish and therefore it was included in the original EC allergens legislation (EC 2003/89; Annex IIIA), which became effective in 2005.

Almost all fish allergy involve parvalbumin, a protein found in the muscle of most fish; levels of parvalbumin have been reported to vary widely across species.

(Ref: <http://foodallergens.ifr.ac.uk/>).

FISH-CHECK ELISA KITS are designed for the measurement of parvalbumin content; for fresh COD, the assay has a nominal range of 5-125 mg/kg (PPM) of cod tissue (Limit Of Detection <1.0 PPM).

The reactivity of other species - see below - and of processed foods may be somewhat different.

Food samples are firstly made homogeneous by grinding/chopping etc; a test portion of e.g. 0.4g is added to 3.5ml of extraction buffer and shaken for 2-3 minutes to extract parvalbumin.

The extract is centrifuged or allowed to settle prior to dilution (1/5) and performing the ELISA (20 + 20 + 20 min. incubation times).

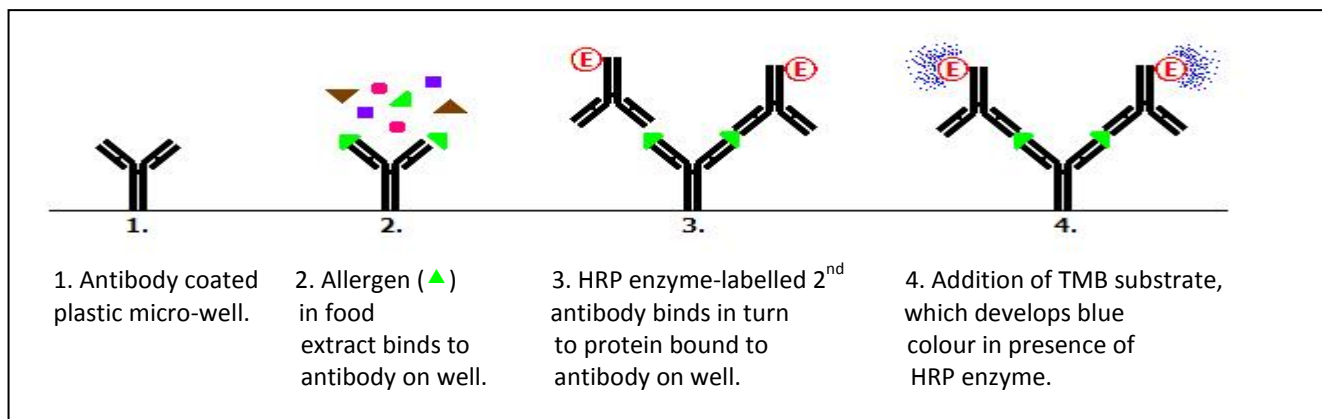
LA013 Flyer FISH v03

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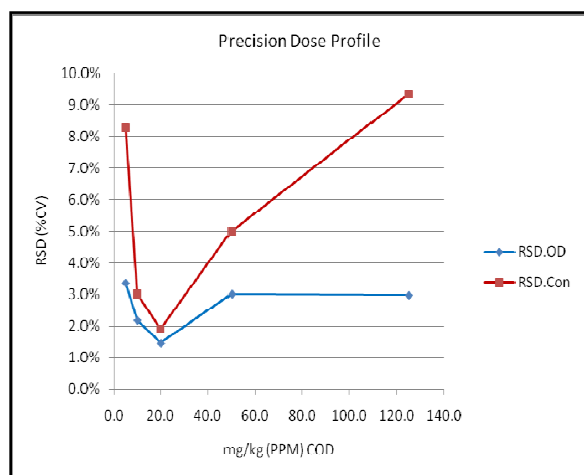
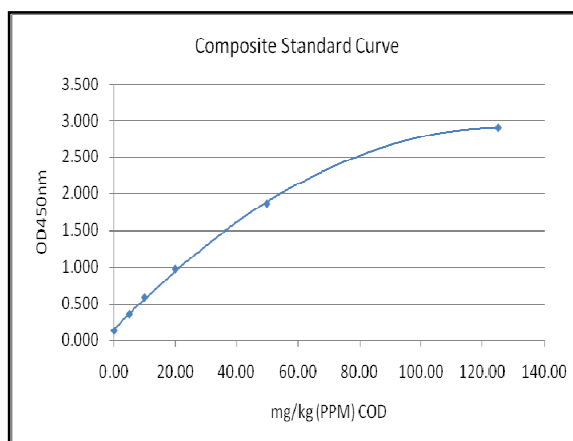
DIAGRAM OF ALLERGEN-CHECK ELISA METHODS:



Bio-Check (UK) FISH-CHECK ELISA

ASSAY VALIDATION DATA

Repeatability, (in-house) Reproducibility and Robustness were assessed by performing ten assays (two operators) and three lots of assay specific reagents - both within and at the end of their expiry dates - over a period of two months. See composite Standard Curve (right).



The Precision Dose Profile (left) shows a plot of the RSD (%CV) of both OD450nm and Concentration across the Standard Curve.

As all Concentration values are below 10% the valid quantitation range is **5-125mg/kg (PPM) Cod**.

Within Assay RSDs were:

3.0% for OD450nm (including Zero ODs)

5.8% for mg/kg Cod (Standards & Kit Control)

The Range, Average value and Between Assay RSD (%CV) for the diluted Kit Control were:

12.4-14.7mg/kg; 13.2mg/kg & 5.1

The Average Limit Of Detection (Zero + 3 x Std. Dev.) was **0.34mg/kg**.

Signal:Noise ratio (5mg/kg Standard:Zero OD averaged **2.7 x** (range 2.6-3.0)).

Reactivity with different fish species (relative to Cod=100%):

Monkfish	Tuna	Skate	Mackerel	Salmon	Trout
Actinopterygii	Actinopterygii	Chondrichthyes	Actinopterygii	Actinopterygii	Actinopterygii
Lophiiformes	Perciformes	Elasmobranchii	Perciformes	Salmoniformes	Salmoniformes
Lophiidae	Scombridae	Rajiformes	Scombridae	Salmonidae	Salmonidae
0.01%	0.16%	0.23%	0.74%	8.0%	7.2%

Herring	Sea Bass	Pollack	Coley	Whiting	Haddock
Actinopterygii	Actinopterygii	Actinopterygii	Actinopterygii	Actinopterygii	Actinopterygii
Clupeiformes	Perciformes	Gadiformes	Gadiformes	Gadiformes	Gadiformes
Clupeidae	Moronidae	Gadidae	Gadidae	Gadidae	Gadidae
11.6%	25.0%	31.3%	36.9%	50.4%	56.7%

Cross Reactivity with various food commodities:

100% samples of finely ground individual food commodities were tested; all but three were found not to react in the assay. Buckwheat (19ppm), amaranth (8ppm), red lentil (5ppm) & soya (4-5ppm) were found to interfere slightly in the assay. Other commodities listed below were negative in the assay:

Alfalfa	Gelatin (porcine)	Peanut
Almond	Gluten, wheat	Pecan nut
Amaranth	Hazelnut	Pine nut
Apricot kernel	Kamut flour	Pistachio
Barley	Lentil (green)	Potato (powder)
Beef, meat	Lentil (red)	Prawn
Brazil nut	Linseed	Pumpkin seed
Buckwheat	Lupin	Quinoa
Cashew	Macadamia	Rice – White; flour
Cheese, cow's	Malt extract	Sesame
Chick peas	Milk (Bovine) powder	Soya flour
Chicken, meat	Milk (Ovine) powder	Spelt flour
Cocoa powder	Millet	Sucrose/sugar
Coconut (flour)	Mung bean	Sunflower
Corn/maize	Mussel	Teff flour
Egg (white)	Mustard	Walnut
Egg (yolk)	Oats	Wheat, flour, white
Gelatin (fish)	Pea	

ALLERGEN-CHECK & GLUTEN-CHECK ELISAs

PRODUCT SUMMARY

Product Name	Wells	Cat. No.	Product Description
Gluten-Check Including Extraction Kit	96	R6006-2E	Gluten (ó-gliadin) detection by ELISA (LOD <0.5ppm; range 5-110ppm gluten) INCLUDING 1 x A6017 for each 48 wells.
	48	R6005-1E	
Fish-Check Including Extraction Kit	96	R6010-2E	Fish protein detection by ELISA (LOD <1ppm; range 5-125ppm cod) INCLUDING 1 x A6015 for each 48 wells.
	48	R6009-1E	
Fish-Check	96	R6010	Fish protein detection by ELISA (LOD <1ppm; range 5-125ppm cod)
	48	R6009	
Almond-Check*	96	R6018	Almond protein detection by ELISA (LOD <0.25ppm almond; range 0.5-10ppm almond)
	48	R6019	
β-LG/Milk-Check*	96	R6036	Beta-Lactoglobulin (milk/whey) detection by ELISA (LOD <0.1ppm Skim Milk Powder; range 0.2-7ppm)
	48	R6037	
Casein/Milk-Check	96	R6022	Casein (milk/caseins/caseinates) detection by ELISA (LOD <0.2ppm Skim Milk Powder; range 0.8-16ppm)
	48	R6023	
Cashew-Check*	96	R6046	Cashew protein detection by ELISA (LOD <0.2ppm cashew; range 2-50ppm cashew)
	48	R6047	
Crustacea-Check	96	R6026	Crustacea protein detection by ELISA (LOD 1ppb tropomyosin; range 20-400ppb)
	48	R6027	
Egg-Check*	96	R6032	Egg White protein detection by ELISA (LOD <0.2ppm Egg White Protein; range 0.4-8ppm)
	48	R6033	
Egg-In-Wine-Check	96	R6044	Egg White protein detection by ELISA (LOD <0.1ppm Egg White Protein; range 0.5-15ppm)
	48	R6045	
Hazelnut-Check*	96	R6014	Hazelnut protein detection by ELISA (LOD <0.5ppm hazelnut; range 1-40ppm hazelnut)
	48	R6015	
Lupin-Check*	96	R6030	Lupin protein detection by ELISA (LOD <0.5ppm lupin; range 2-30ppm lupin)
	48	R6031	
Total Milk-Check	96	R6040	Casein & β-LG detection by ELISA (LOD <0.1ppm Skim Milk Powder; range 1-27ppm)
	48	R6041	
Mustard-Check*	96	R6012	Mustard protein detection by ELISA (LOD 1ppm mustard; range 2-50ppm mustard)
	48	R6013	
Peanut-Check*	96	R6020	Peanut protein detection by ELISA (LOD <0.5ppm peanut; range 1-30ppm peanut)
	48	R6021	
Pistachio-Check*	96	R6042	Pistachio protein detection by ELISA (LOD <0.2ppm pistachio; range 1-25ppm pistachio)
	48	R6043	
Sesame-Check*	96	R6028	Sesame protein detection by ELISA (LOD 0.2ppm sesame; range 2-20ppm sesame)
	48	R6029	
Soya-Check*	96	R6024	Soya Trypsin Inhibitor detection by ELISA (LOD <1ppm soya flour; range 2-25ppm soya flour)
	48	R6025	
Walnut-Check*	96	R6016	Walnut protein detection by ELISA (LOD <1ppm walnut; range 2-50ppm walnut)
	48	R6017	

* Food sample extracts from these kits are fully compatible and can be used in any other of the marked * ELISAs.

NOTE: The LOD & Range data given above refer to PPM of the commodity itself and - unless indicated otherwise - NOT PPM of protein, for which the equivalent values are substantially lower.

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