

Performance Verification of an ELISA-based Assay Milk in Chocolate Matrices

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Objective

The “Big Eight” Food allergens mainly includes Fish, Crustaceans, Egg, Milk and Wheat. These allergy are known as IgE- Mediated Food allergies, causing the immune system to react abnormally when exposed.

Due to the proteins present in cow's milk, user with milk allergies normally associated with indigestion and other conditions. The estimated prevalence in developed countries ranges from 0.5% to 3% at the age of 1 year old.

In the midst of manufacturing dark chocolate or milk chocolate , milk is the most commonly used ingredient. There is a possibility with small quantity of the milk contaminated in dark chocolate even when milk is not used as an ingredient.

In this study, four different type of dark chocolates that was declared differently by their manufacturer ; dark chocolate that was declared as dairy-free, “do not contains milk”, “ traces of milk” and “milk used as one of the ingredients” was analyzed. Additionally, milk chocolate is used as a positive control.

Methods

Dark chocolate with different declaration labels was purchased. They are declared as either dairy free, “do not contains milk”, “ traces of milk” or “milk used as one of the ingredients”.

All of the dark chocolate are tested against our AgraQuant Milk ELISA test kit to get a quantification of milk protein presence. The non-detectable matrices are further fortified with low level ppm and high level ppm of milk. Matrices that are tested positive was prepared in serial dilution to confirm is of true positive.

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AgraQuant®
 Allergen ELISA Test Kits



Results and Conclusion

ELISA Results

Type of chocolate	Labels	Result
Milk Chocolate	Contain 20.5% milk	>10
Dark Chocolate	Contain Milk	>10
90% dark chocolate	May contains milk	<LOD
Dark chocolate protein bar	Does not contain milk but facility may contain milk	<LOD
Dark chocolate Protein Bar (dairy free)		< LOD

Spiked ELISA Results

Matrix	Neat	Low Level	Recovery %	High	Recovery %
90% dark chocolate	<LOD	0.38	95	1.22	101.7
Dark chocolate protein bar	<LOD	0.43	107.5	1.24	103.3
Dark chocolate Protein Bar (dairy free)	<LOD	0.36	90	1.30	108.3

Result:

Milk chocolate was used as the positive control to test against AgraQuant® Milk ELISA. It was found to be >10 ppm. On the other hand, Dark chocolate containing milk are found to be >10 ppm while others were found to be <LOD.

These ND dark chocolate samples were further spiked with low and high ppm of milk allergen. The average recoveries of these samples were between 80% -120%

Conclusion:

The results correlate very well with the labelling on the packaging. This gives milk allergy consumers a peace of mind to purchase the chocolates off the shelves in the supermarket as allergen labelling gives very reliable information.

Significance: The AgraQuant® Milk Allergen offers a rapid and reliable tool for testing milk allergens in complex matrices like dark chocolate

Protocol

- 1 • Weigh 0.5 g homogenate in a 50 mL centrifuge tube and add 10 mL extraction buffer. Vortex until all the solid is suspended
- 2 • Incubate the suspension for 15 minutes
- 3 • Centrifuge samples for 10 minutes at 2000 g to obtain a clear supernatant. If there still are particles in the supernatant, filter it and collect the filtrate.
- 4 • Samples are ready for testing.