Simplifying the Detection of Egg Allergens in Heat-Processed Foods

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INTRODUCTION

Egg allergies are among the most common food allergies, affecting around 10% of infants[1], and although there is a reduced prevalence with age the allergy can persist into adulthood. Egg proteins which have undergone heat-treatment have a modified protein structure, making it difficult for most current egg ELISAs to detect them adequately. These modified proteins are still capable of eliciting a serious allergenic reaction in a proportion of children with egg allergies[2].

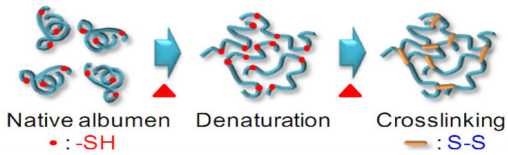
Background

The results of a 2018 international proficiency trial looking at processed egg in a cooked biscuit (0.66% egg white powder) supplied by Fapas highlighted the fact that not all egg ELISA kits were able to accurately quantify an egg sample which had undergone heat-processing.

FAPAS QC MATERIAL DATA SHEET	T27214QC
Matrix	Cooked Biscuit
Weight / Volume of Contents	30 g

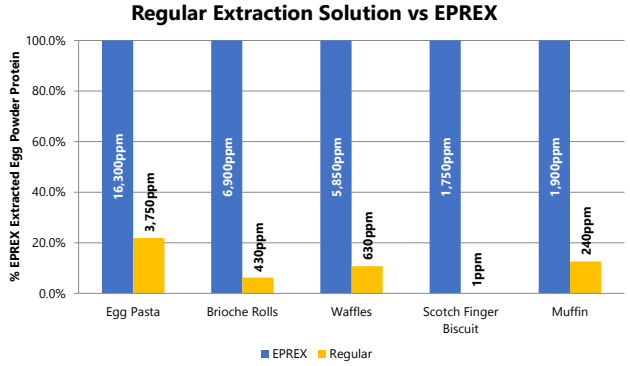
Quantitative Results				
Analyte	Assigned Value, x_a	Range for $\|z\| \leq 2$	Units	No. of data points producing x_a
R-Biopharm - Ridascreen Fast Ei/Egg protein (R6402)	134	67 - 201	mg/kg	20
Morinaga - Egg Protein ELISA Kit	3399	1700 - 5099	mg/kg	4

This marked underestimation by most kits used in the proficiency test is likely due to the denaturation and cross-linking (example below[3]) of the egg proteins. This can lead to entanglement with other proteins present in the biscuit mixture during baking, causing the egg proteins to become insoluble or rearranging them in a way which obscures the assay's target epitopes. The Morinaga kit already addresses this issue by using high heat and reducing agents to solubilize processed proteins.



The ESEGGPR-48 Assay

ELISA Systems has developed a new Processed Egg kit (ESEGGPR) to combat this problem. The kit comes with a modified extraction solution (EPREX) for the effective extraction of processed egg proteins. Below is a comparison of the new EPREX with an extraction solution found in a regular egg kit.



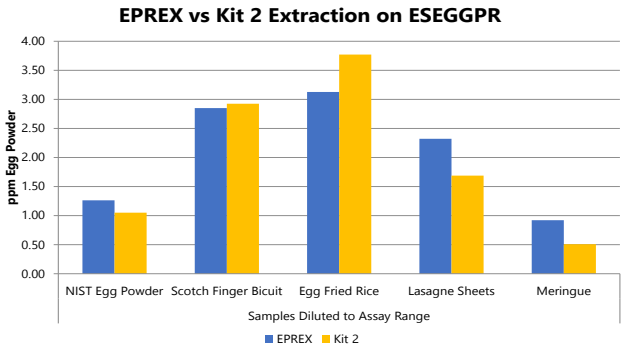
Comparison of Kits

The main drawback of current processed egg kits is the complex methods required for sample extraction and preparation. The ESEGGPR kit solves this by keeping extraction as a simple one-step process.

	ESEGGPR	Kit 1	Kit 2
Easy preperation of extraction buffer?	**Yes** (dilution only)	**Yes** (dilution only)	**No** (dilution of buffer, alkali dilution of buffer additive, pH adjustment of resulting buffer, dilution of extra additive)
Simple extraction method?	**Yes** (15 minutes @40°C)	**No** (Boiling of sample required, 10 minutes @100°C)	**No** (Pre-heat extraction solution, preperation of additional additives, 10 minutes @60°C)
Samples ready to use after extraction?	**Yes**	**No** (additional dilution in second buffer required)	**Yes**
Simple sample dilutions?	**Yes** (use extraction buffer)	**No** (use of third buffer required)	**No** (extra buffer preperation required)
Standards ready to use?	**Yes**	**No** (Series 1/2 dilutions in third buffer six times, 0ppm standard is third buffer)	**Yes**
Conjugate ready to use?	**Yes**	**Yes**	**Yes**
Total incubation time?	**40 minutes**	**110 minutes**	**50 minutes**

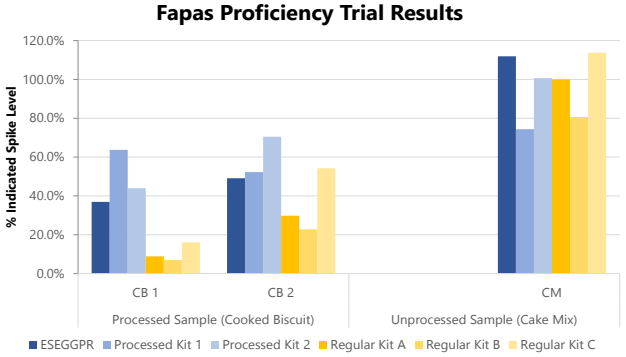
Extraction Comparison

Through testing we have compared the EPREX egg extraction with a currently available processed egg kit's extraction (Kit 2), and found that they perform similarly when run on either kit's components.



Fapas Data

Fapas proficiency data has also been collated to demonstrate ESEGGPR's ability to detect both processed and unprocessed forms of egg when compared with other kits used in these trials.



SUMMARY

Due to egg's ability to elicit an immune response even after undergoing heat-processing, and the importance of accurate quantification for risk assessment, a new processed egg kit has been developed by ELISA Systems. This kit is able to accurately detect samples which have been heat treated via a simple and easy to use method, whilst retaining the ability to detect native egg samples in unprocessed foods.

REFERENCES

1. Peters RL, Koplin JJ, Gurrin LC, DharmageSC, Wake M, Ponsonby AL, Tang MLK, Lowe AJ, Matherson M, Dwyer T, Allen KJ. J Allergy Clin Immunol. 2017 Jul 140(1):145-153. doi: 10.1016/j.jaci.2017.02.019
2. Yonkof JR, Mikhail IJ, Prince BT, Stukus D. The Journal of Allergy and Clinical Immunology: In Practice. 2021 Jan 1;9(1):283-9. doi: 10.1016/j.jaip.2020.09.059
3. Chang JW, Wang CG, Huang CY, Tsai TD, Guo TF, Wen TC. 2012 Apr SPIE.org