

FAPAS REFERENCE MATERIAL DATA SHEET	TYG001RM, TYG002RM
Matrix	Cake mix
Weight / Volume of Contents	2 x 30g
Description of material: Commercial cake mix manufactured to be gluten-free, egg-free and milk-free, blended with ingredients known to contain gluten, egg and milk. Gluten was added as gluten powder. Egg and milk were added as known amounts of total ingredients but the egg and milk content of those ingredients is not fully traceable.	
The reference values and uncertainties are listed in the table on the second page.	

Date reference values were generated	21/10/2016
Reference values are valid until	20/10/2020
Recommended storage conditions on receipt	Cool ambient or refrigerated, dark
This material was approved on behalf of Fapas by	Mark Sykes

Notes

- Mix the reference material thoroughly before taking a representative analytical sample. It is intended to be used as a single-analysis sample (plus confirmation) for analytical quality control purposes. The recommended analytical sub-sample size is 0.5 g (dependent on ELISA kit used).
- This is a reference material, not a certified reference material. It is designed to be a relative response pair of reference materials, not absolute reference materials.
- This reference material has been produced according to the principles of ISO 17034.
- The reference values have been derived from the results consensus of ISO 17025 accredited laboratories taking part in an interlaboratory proficiency test, using a variety of ELISA methods. The traceability is inherent in the accreditation status of the results used.
- The Expanded Uncertainty U corresponds to a confidence level of about 95%. U has been derived from the observed standard deviation of the consensus data (the major component) plus contributions from homogeneity and stability studies.
- The stability of the reference material has been established from a formal study. The stability components combine long term (ideal storage) and short term stability (transportation) conditions. The validity date may be extended if supporting data becomes available.
- The previous validity date was 20/10/2018.

How to use these reference materials

The majority of laboratories undertaking allergens analysis do so by ELISA kit. The results of one ELISA kit cannot be compared directly to another. This reference material comes in two parts so that a relative comparison can be made between methods [1, 2].

Reference values and their uncertainties are provided for each material/ELISA kit combination. The reference values are valid only for the named ELISA kit and this does not constitute recommendation, endorsement or promotion for that ELISA kit manufacturer. The ELISA kits listed are simply a reflection of those kits most widely used by laboratories that contributed to the interlaboratory comparison.

For beta-lactoglobulin (BLG), there are no reference values for any ELISA kit. ELISA kits of any type reporting BLG appear to have a non-linear response (the observed ratio of test materials does not match the preparation ratio). It is not recommended to use this reference material for the assessment of methods reporting BLG.

Where an ELISA kit (or other method) is not listed, there is no defined reference value available.

The ratio of allergens in the two materials will provide an indication of the expected linear response for any given method. This allows the user to estimate the relative response factors for their own method against the reference materials and the values defined for the named ELISA kits.

[1] Owen, L, Gilbert, J, Proficiency testing for quality assurance of allergens methods, *Anal Bioanal Chem*, 2009, 395:147-153

[2] Sykes, M, Anderson, D, Parmar, B, Normalisation of data from allergens proficiency tests, *Anal Bioanal Chem*, 2012, 403:3069-3076

RM	TYG001RM				ratio	TYG002RM			
	Ref value	<i>U</i>	Units	n		Ref value	<i>U</i>	Units	n
<u>Gluten</u>									
R-Biopharm - Ridascreen Gliadin (R7001) or R-Biopharm - Ridascreen Fast Gliadin (R7002)	14.4	1.7	mg/kg	95		28.2	2.4	mg/kg	103
Preparation target value (gluten powder)	15		mg/kg			30		mg/kg	
Ratio for Gluten (preparation)					0.50				
Ratio for Gluten (measured)				118	0.53				
<u>Egg</u>									
R-Biopharm Ridascreen Fast Ei/Egg protein (R6402)	83.5	9.5	mg/kg	23		27.5	3.8	mg/kg	23
Neogen - Veratox for Egg Allergen (8450)	103	16	mg/kg	10		34.1	3.4	mg/kg	11
Preparation target value	NA					NA			
Ratio for egg (preparation)					3.0				
Ratio for egg (measured)				40	3.1				
<u>Egg white protein</u>									
Neogen - BioKits Egg Assay Kit (902072T)	32.6	7.0	mg/kg	6		10.7	1.8	mg/kg	6
Preparation target value	NA					NA			
Ratio for egg white protein (preparation)					3.0				
Ratio for egg white protein (measured)				13	3.1				
<u>Milk</u>									
R-Biopharm - Ridascreen Fast Milk (R4652)	28.6	4.8	mg/kg	12		90.6	17.7	mg/kg	12
Neogen - Veratox for Total Milk Allergen (8470)	101	18	mg/kg	18		324	64	mg/kg	18
Preparation target value	NA					NA			
Ratio for milk (preparation)					0.33				
Ratio for milk (measured)				32	0.34				
<u>Casein</u>									
R-Biopharm - Ridascreen Fast Casein (R4612)	26	7	mg/kg	10		72	9	mg/kg	8
Preparation target value	NA					NA			
Ratio for Casein (preparation)					0.33				
Ratio for Casein (measured)				18	0.33				

Ref value is the reference value, *U* is the expanded uncertainty of the reference value, n is the number of datapoints contributing to the reference value.