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validation report for
GMO Extraction Kit

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Contents

2. Purpose

The present report is intended to define the methodology used to validate the extraction and quantification stage for whole DNA obtained from raw materials, foodstuffs and feeds using the GMO Extraction Kit.

3. References

Guideline UNE-EN ISO/IEC 17025:2005
Guideline UNE-EN ISO/IEC 21571:2005

4. Definitions

Validation: Confirmation by examination and the provision of objective evidence to demonstrate compliance with certain requirements for the expected specific use (ISO 17025)

Repeatability: Precision under conditions in which the results of a measurement are obtained using the same method, by the same operative, and using the same measurement instrument.

Reproducibility: Precision under conditions in which the results of a measurement are obtained using the same method and same measurement, but with different operatives and different measurement instruments.

Precision: The degree of concordance between the results of independently obtained measurements under the conditions established.

Recovery tests: Tests that involve adding a known quantity of DNA obtained from a standard sample to a matrix in order to verify that the DNA added is recovered after the extraction process used. The aim of such tests is to determine the absence of inhibition during the extraction process.

5. Introduction

The GMO Extraction kit has been designed to obtain whole genomic DNA from complex samples such as foodstuffs and feeds. The DNA obtained can be used to perform PCR reactions or in other molecular biology procedures. The main specifications or characteristics of the kit are as follows:

Table 1: Recommended quantity of sample to be analysed, depending on its nature.

Product Type	Quantity of Sample
Seeds	20g
Flour, semolina, baked goods, meat products, fish, snacks, manufactured products, etc.	10g
Feed and soy beans	10g
Cocoa, soy flour	5g
Oil, fats, butter	10 mL
Dairy products, fruit juices, ice creams, alcoholic beverages	10 mL

Expected yield of DNA

The extraction yield depends on the degree of processing of the matrix analysed. Thus, those samples submitted to more extreme physicochemical processes will provide a lower yield of DNA after the extraction process. All samples were selected between a wide and representative range of commercial products. The following table shows the expected DNA yields depending on the analysed samples:

Table 2: Expected DNA yields depending on the type of sample analysed, together with recommended elution volumes

Product Type	Expected yield ng/µL
Starch, corn flour	0 - 10
Sauces	0 - 25
Flavours, colourants	0 - 25
Soups, concentrates	0 - 25
Flour, pasta	50 - 100
Semolinas	25 - 100
Seeds	50 - 100
Sugars	0 - 10
Meats products, fish, coated products, Salads, rice dishes,	50 - 100
frozen food	25 - 100
Baked goods	25 -100
Preserves	5 - 50
Soy flour	50 - 100
Cocoa derivatives	0 - 50
Soy lecithin	0 - 10
Oils, fats, butter	0 - 10
Alcoholic beverages	0 - 10
Snacks	5 - 100
Breakfast cereals	5 - 100
Feed	50 - 100
Soy drinks	25 - 100
Dairy products, fruit juices, sweets	0 - 100

6. Validation Assay

This report summarises the results obtained during validation of the DNA extraction and quantification stage when using the GMO Extraction Kit.

6.1. Repeatability and reproducibility

Five different matrices, extracted by two different operatives, were used to assess the reproducibility and repeatability of the extraction process. Each operative performed 5 replicates for each matrix, varying the extraction day and the equipment and reagents used. The extraction protocol recommended by the kit's manufacturer was followed at all times. The following table shows the results obtained:

Table 3: Results for the repeatability and reproducibility assay for the GMO Extraction Kit.

Results for limit of quantifiication (LO Qabs)					
MATRIX		**No. replicates**	**Mean (ng/µl)**	**Deviation**	**CV**
Soy Drink	**Operative 1**	5	26.68	5.43	18.99 %
	Operative 2	5	25.80	3.91	15.14 %
Cooked Sausages	**Operative 1**	5	177.20	42.99	24.26 %
	Operative 2	5	148.20	24.51	16.52 %
Baby food	**Operative 1**	5	68.98	14.8	21.45 %
	Operative 2	5	76.58	15.05	19.66 %
Feed	**Operative 1**	5	180.2	40.43	22.44 %
	Operative 2	5	140.2	29.27	20.07 %
Corn flour	**Operative 1**	5	111.02	24.18	21.78 %
	Operative 2	5	116.98	21.08	18.02 %

The acceptance criteria for deviation and CV comes from a wide statistical study in which, the differences between operators (reproducibility) and repeatability, measured as CV, should be less than 25%.

6.2. Matrix effect

Three different samples included in each of the categories in the Codex alimentarius were selected to assess the effect of the matrix on the yield of the extraction process. For some categories, the validation was performed on various sub-categories due to the heterogeneity of the matrices included therein.

www.codexalimentarius.net/gsfaonline/foods/index.html

Table 4: Matrices tested to validate the matrix effect.

Product Type		
Yoghurt with cereals	Macaroni	Brown Sugar
Chocolate milkshake	Corn Starch	Honey
Powdered milk	Breakfast cereals	Oregano
Oil	Soy lecithin	Mayonnaise
Butter	Baguette	Chicken soup
Lard	Rich Tea biscuits	Beef and vegetable baby food
Strawberry ice lollipop	Crackers	Follow-on baby milk
Lemon slush	Meatballs	Starter milk
Mandarin orange sorbet	Pâté	Peach juice
Mixed vegetables	Minced meat	Coffee
Soy beans	Hake	Beer
Fresh pineapple	Shrimps	Fried maize
Cocoa cream (Nutella)	Surimi	Potatoes
Sweets	Potato omelette	Japanese appetiser
Nesquik	Egg custard	Stir-fried mushrooms
Wheat flour	Eggs	Spinach lasagna
Corn	Sugar	Prepared rice

A quantity of DNA in accordance with the acceptance criteria established in Table 2 was obtained in all cases. Better yields were obtained in some cases.

The quality of the DNA obtained was assessed by spectrophotometry and PCR amplification. The ratio of absorbance at 260 nm and 280 nm was used to assess the purity of DNA. The acceptance range, for extractions with yields higher than 10 ng/µl, was 1,7-2,0. Recovery tests were performed for all matrices where the yield was less than 10 ng/µl.

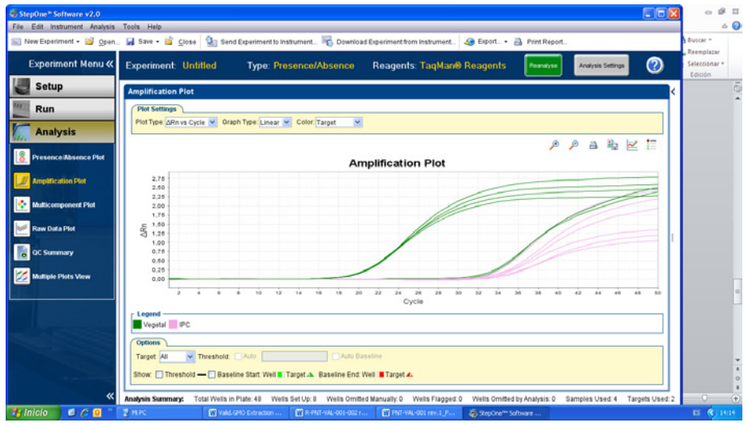
6.3. Evaluation of DNA quality

All the DNA samples extracted were amplified using the plant/IPC system in the TaqMan® GMO screening kit to confirm that the extractions performed were of sufficiently high quality for subsequent real-time PCR.

Furthermore, amplification with the plant/IPC system in the TaqMan® GMO screening kit, allows the presence of inhibitors in the extracted DNA samples to be ruled out. Amplification of the internal positive control (IPC) was detected in all samples analysed, thus ruling out the presence of inhibitors in the DNA extract.

The following chart shows an examples of some of the DNA samples obtained after extraction. The concentration of all samples was adjusted to 10 ng/µl prior to amplification.

Chart 1: Evaluation of the quality of the DNA obtained.



6.4. Recovery Tests

To perform the recovery tests, a total of nine extractions were performed with each product for which the yield obtained was less than 10 nq/µl: three of these extractions were performed using the "test" sample, three using the reference standard (1g of wheat flour) and three using the "test" product spiked with 1g of reference standard (wheat flour).

The aim of these tests was to check that the DNA added to the test product (standard) was recovered during the extraction. Thus, the quantity of DNA obtained upon extraction of the product spiked with reference standard (B) was compared with the quantity obtained upon extraction of the standard (A) and test product (C).

The results obtained can be found in the following table. Statistical analysis of these results shows that the recovery was satisfactory in all cases.

Table 5: Results for the recovery tests.

Recovery tests				
MATRIX	**EXTRACTION**	**Absorbance (ng/µl)**		
		Replica 1	**Replica 2**	**Replica 3**
Wheat flour (reference)	A	23.2	19.8	18.8
Oil	B	21.6	23.5	23.5
	C	1.2	0.9	2.6
Butter	B	25.3	17.8	21.5
	C	2.8	1.3	2.1
Lard	B	23.5	25.6	19.8
	C	2.5	1.8	2.3
Strawberry ice lollipop	B	21.5	19.8	22.6
	C	0.8	2.0	1.5
Lemon slush	B	23.5	25.4	20.4
	C	0.8	1.5	1.3
Sweets	B	20.5	18.9	24.6
	C	0.8	1.1	0.9
Corn starch	B	30.5	32.6	28.4
	C	6.9	8.2	5.4
Soy lecithin	B	26.5	27.9	22.9
	C	6.2	3.8	4.3
Sugar	B	20.8	22.5	23.6
	C	0.9	0.6	0.7
Honey	B	26.8	27.3	25.1
	C	3.5	4.6	4.9
Beer	B	20.6	18.4	25.6
	C	0.3	0.2	0.5
Fried maize	B	26.5	33.2	21.4
	C	6.9	5.7	7.5

7. Conclusion

The data reported confirm that the GMO Extraction Kit applied to different food and feed samples produces DNA of suitable quality and quantity for subsequent PCR based detection applications, like genetically modified organism detection or quantification.