

**Nucleic Acids Extraction Kits** 

Selection Guide





Developments in real-time PCR based detection and quantification methods have significantly contributed to increase the efficiency of quality control in food industry. Control laboratories are daily working with qPCR assays for detecting pathogens, spoilage microorganisms, GMO, allergens or for preventing frauds. Historically, suppliers stressed a lot the performances of the detection kits, often forgetting that in agri-food applications DNA extraction has an equivalent importance in the quality of results.

DNA-based methods, such as polymerase chain reaction (PCR), are highly specific, reproducible, sensitive and characterized by high discriminatory power, rapid processing time and low costs, they are strongly limited by the presence of inhibitors in food. PCR inhibitors, such as polysaccharides, humic acids, particularly abundant in food samples are not completely removed during classical extraction protocols remaining as contaminants in the final DNA preparations. The inhibitor compounds can interfere with the reaction at several levels, leading to decreasing and even to complete inhibition of DNA polymerase.

Thus, the DNA-based methods are highly dependent on the DNA extraction and purification techniques. In particular, samples requires:

- a stringent extraction and purification strategies that ensure efficient recovery of nucleic acid and removal of the numerous compounds inhibiting PCR assay.
- extraction methods capable to recover DNA fragmented/degraded by changes in pH or temperature(cooking, sterilzation, smoking etc.) foods are often subjected to during the production process.
- when heterogeneous, possibility to extract from large amount of material
  to ensure representativeness. This is especially valid when testing for
  contaminants like GMOs, allergens and undeclared meat/vegetable
  species.

Nucleic acids based analysis methods and their standardization are the core of **Generon** R&D and sales activities as we aim to be the most reliable partner in this field for food and beverage industry.

**ION** is the portfolio of DNA extraction products developed by **Generon** to exploit at best Real-Time PCR and Droplet Digital PCR, three kits with different profiles to offer the customer the most convenient solution to fit the technical demands.

- Fast Food DNA extractor the rapid solution specifically developed for crude and fast extraction of bacteria DNA after media broth enrichment.
- ION-Spin DNA Prep One-for-All CTAB based kit for the extraction of DNA from small samples containing PCR inhibiting compounds
- ION-Force DNA extractor FAST CTAB based kit for the extraction of DNA from large samples when representativeness is of paramount importance. Ion-Force DNA Extractor FAST is used at the Italian National Reference Lab for GMO as it allows reproducible DNA extractions from matrices the other available commercial kits are not able to perform on, giving poor and uneven DNA yields.

Table 1 – Fit-to-purpose of the different extraction kits for downstream applications

	GMO Analysis	Allergen Analysis	Microbiology Analysis	Ingredient Authenticity
Fast Food DNA extractor	**	*	****	*
ION-Spin DNA Prep One-for-All	***	***	***	***
ION-Force DNA extractor FAST	***	****	*	****

Table 2 – Technical characteristics of the different extraction kits

	Speed	Versatility	Sample Size	Purity
Fast Food DNA extractor	****	*	**	*
ION-Spin DNA Prep One-for-All	***	***	**	****
ION-Force DNA extractor FAST	*	***	***	****

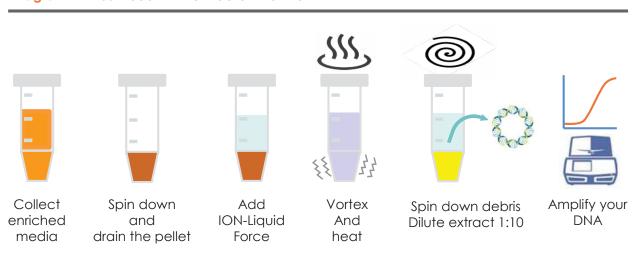


## Fast Food DNA extractor (EXD009)

It is the kit developed by **Generon** for extracting DNA of microorganisms as fast as possible. The extreme ease of use fits perfectly with the extraction of DNA from bacterial pellet derived from enrichment broth. Fast Food DNA extractor is also validated for extracting DNA from feed ingredients to screen the presence of GMO via qPCR over a 0.1% threshold.

The extract is very crude therefore in case the food matrix tested contains a lot of PCR inhibitors we suggest to upgrade to ION-Spin DNA Prep One-for-All.

Diagram 1 – Fast Food DNA extractor workflow



The sampling procedure determines the "representativeness" of a result, whereas quality and quantity of the analytes may vary depending on the sample preparation. Sampling and sample preparation are thus crucial steps in the process of contaminants DNA detection.

The limit of detection of the analytical method as a whole is determined, not by the most sensitive part of the procedure, but by the least. In most cases this is the sample size. A sample has to be representative of the batch/lot of the product from which it was taken, and the sample plan and sample size have to meet statistical requirements with respect to homogeneity and threshold limit up to which the result should be reliable.



## ION-Force DNA Extractor FAST (EXD001)

It is the kit planned to maximize the yield and purity of the DNA extracted from simple or complex matrixes (rich in PCR inhibitors). It is an essential system to obtain repeatable and reproducible data in molecular detection from samples containing minimal traces of contaminants DNA, thanks to the following advantages:

- 1-4-ALL APPROACH allows extraction from both vegetal or animal matrices
- SCALABLE PROTOCOL allows extractions from representative sampling units
- FRAGMENTED DNA RECOVERY allows extractions from processed matrices
- PROGRESSIVE CLEAN-UP allows removal of PCR inhibitors present in matrices
- COMPLIANCE based on CTAB extraction according to ISO indications

Diagram 2 – ION-Force DNA extractor workflow



**Table 3** – List of matrices successfully extracted using ION Force DNA Extractor and ION-Spin DNA Prep One-for-All directly or after media enrichment for downstream microbiological analysis.

Cereals	arains	and	millina	derivatives	Toppings
	9				

Soy beans and processing derivatives Biscuits and Snacks

Raw and refined vegetable oils Extruded food and feed

Starches and sugars Freeze-dried food

Feed and MBMs Soy based food

Lecithin liquid and powder Flavors and spices

Eggs and derivatives Salad dressings

Fresh Vegetables Würstel and cold cuts

Dried or canned vegetables Honey

Candy fruits Dietary integrators

Fruit juices Baby food and infant formula

Liquid and powdered milk Swabs

Milk, dairies and ice-creams Water and wastewater

Meat (raw or cooked)

Pasta and pasta preparations

Beverages Tomato based sauces

Cakes and chocolate Textiles

## ION-Spin DNA Prep One-for-All (EXD018)

It is a miniaturized version of the parent kit ION-Force and shares the same workflow shown in diagram 2. This kit allows extractions only from small amount of material (up to 1 gram) but needs minimal laboratory equipment: a small heating block and a micro-centrifuge. ION-Spin DNA Prep One-for-All is the ideal kit when extracting microbiological samples from "difficult" matrices and offers like ION-Force the following advantages:

- 1-4-ALL APPROACH allows extraction from both vegetal or animal matrices
- SCALABLE PROTOCOL allows extractions from representative sampling units

  FRAGMENTED DNA RECOVERY allows extractions from processed matrices
- PROGRESSIVE CLEAN-UP allows removal of PCR inhibitors present in matrices
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The overall testing success in molecular methods depends on DNA isolation. For many reasons different matrices and also different samples yield different amount of DNA extract per gram. DNA Quantity defines representation, and the contribution to sampling error. It's therefore convenient to measure the quantity of the DNA obtained after the extraction and to normalize the quantity of DNA loaded in a molecular reaction because normalization:

- Provides uniformity in testing
- To comply with the sampling scheme
- To comply with validated process/assays

Enhances robustness of testing, higher success rate, hence, in the long run reduces cost, and on the average may improve turn around time. In fact, although qPCR enables in-assay normalization, extracts normalization allows for semi-quantitative analysis.



Promega Quantus<sup>™</sup> (ACC2037) and the associated Quantifluor<sup>™</sup> dsDNA (ACC2038) kit are the method of choice at **Generon** for DNA quantitation.



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