# ION Force
## DNA Extractor Fast

**Cat. #** EXD001

READ SAFETY INFORMATION AND DISCLAIMERS BEFORE USING THE KIT

## 1 - Introduction

ION Force DNA Extractor FAST is a DNA extraction kit developed to fulfil the demands of the molecular biologist working in the food and feed diagnostics field. ION Force is indicated for complex matrices, where DNA purification may result quite difficult due to several inhibition substances. Generon developed the product using premium quality molecular biology reagents and provide the customer a very flexible protocol allowing DNA recovery from a wide range of matrices. All reagents comply with ISO 21571:2005/Amd 1:2013 technical specifications. ION Force combines the CTAB sample preparation with a silica-based microspin purification column; this allows the extraction of DNA from simple and complex matrices maximizing the yield and purity of the DNA.
The resulting DNA can be used immediately for downstream molecular applications such as Restriction Endonuclease Digestions, Real-Time PCR, Southern Blots or Droplet Digital PCR. Alternatively, the DNA can be stored at 4°C for few days or frozen.
This User Manual reports protocols covering most of the applications but also special procedures for peculiar matrices like soy lecithin. The protocol is scalable and allows to process up to 20 grams of starting material to maximize sample representation. The starting sample must be properly ground and homogenized before starting DNA extraction. Ion Force provides also a fast and reliable purifying genomic DNA (gDNA) method from bacteria broth (after enrichment step), filters and swabs, molds/fungi and protozoa, featuring a simple protocol that can be completed in less than 15 minutes after preparing lysates.
The ION Force DNA Extractor FAST contains sufficient reagents for 100 extractions.

**It is strongly recommended to read the entire protocol before starting the extraction.**

## 2 – Kit Components

| Product | Quantity |
|---|---|
| Solution A | 4 x 500 ml |
| Buffer E | 1 x 100 ml |
| Columns | 100 units |
| Collection Tubes | 100 units |
| Buffer T | 3 x 250 ml |
| Buffer P concentrate | 1 x 50 ml |
| Solution D | 1 x 20 ml |

Buffer P concentrate should be reconstituted by adding 200 ml ethanol (purity > 98%) to obtain 250 ml of ready to use solution. Label the bottle after adding it.

**Spare components available:**

| **Cat. #** | **Description** | **Format** |
|---|---|---|
| **EXD003** | ION Force DNA Extractor SOLUTION A | 500 ml |
| **EXD004** | ION Force DNA Extractor SOLUTION D | 20 ml |
| **EXD005** | ION Force DNA Extractor BUFFER T | 250 ml |
| **EXD006** | ION Force DNA Extractor BUFFER P | 50 ml |
| **EXD007** | ION Force DNA Extractor COLUMNS | 100 units |
| **EXD013E** | ION Force DNA Extractor Buffer E (Purification step) | 100 ml |

## 3 – Material required but not supplied

- N-Hexane (optional, only for fluid lecithin).
- Ethanol (purity > 98%).
- Tubes 15 and 50 ml.
- Moulinex homogenizer or equivalent.
- Plastic spoons.
- Technical balance, sensitivity: 0.01 g.
- Thermal Water Bath or Block heated up to 85°C ± 2°C.
- Centrifuge with rotors for 1.5-2.0 ml microtubes and 50 ml tubes (range within 500–14000 rpm).
- 1.5 and 2.0 ml microtubes.
- 10 ml syringes without needle* and cellulose acetate filters 0.45 µm pore size.
- DNA Extraction VACUUM BOX (Cat. # EXD010-P) + Vacuum pump (Cat. # ACC2908).
- Adapters for BOX VACUUM (Cat. # EXD010-A, 6 packs)*
- Syringes (10 ml Luer-lock reservoir, Cat. # ACC2911).
- Graduated cylinder 50 ml.
- Pipette sets.
- Pipette tips (barrier).

Each step of samples preparation (grinding, transferring, weighing, etc.) must be done according to GLP (Good Laboratory Practice) It is recommended to possibly use disposable plasticware to minimize risks of cross-contamination.

*NOTE: Ion Force batches delivered from February 2021 may no longer require the EXD010-A adapters (Adapters for Vacuum Box), because they have been entirely replaced by the new ACC2911 syringes (10 ml Luer-lock reservoir).

## 4 – Short-term stability, Storage and Shelf life

Once received, the kit must be stored at room temperature. Reagents stored at this temperature can be used until the expiration date indicated on the packaging.

When exposed to temperature 4-8°C, Solution A may flocculate and form a precipitate. When resolubilized, the initial presence of the precipitate does not compromise the functionality of Solution A and, therefore, of the entire extraction kit. The precipitate can be easily and completely resolubilized by heating the Solution at 37°C (using an incubator or a water bath) and gently shaking.

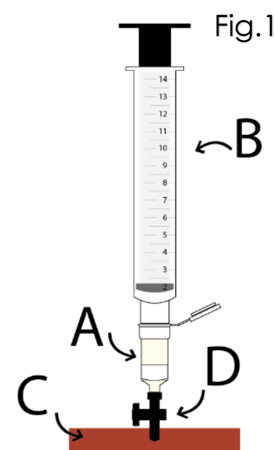
# 5 – Extraction protocol

Details of the extraction procedure are depicted below. We suggest to use the 20-position vacuum box chamber (EXD010-P) allowing fast parallel sample processing.

1. Grind and/or homogenize the sample. Transfer into a 50 ml tube the requested quantity as indicated in Table 1
2. Add 20 ml of Solution A to the sample*.
   ***For granular lecithin and fatty matrices:** add to the sample 20 ml of n-Hexane. Shake then add 10 ml of Solution A. Again, shake until lecithin is completely dissolved and proceed according to the step 4 of this protocol. The sample has to be processed at room temperature instead of 85°C.
3. Mix well by vortexing and incubate 1 hour at 85°C ± 2°C. Shake 2-3 times during the incubation period.

**Note:** for some matrices the use of proteinase K may increase the yield in DNA. When using Generon Proteinase K (EXD011) add 40 µl/g of reconstituted enzyme solution to the extracting sample and incubate 60' at 65°C.

4. Centrifuge the sample (speed 6000<RPM<10000) for 10 minutes.
   **Note: if a centrifuge with rotors for 50 ml tubes is not available, transfer 2 ml of the processed sample in a 2.0 ml microtube and centrifuge at 10000 rpm for 10 minutes.**
5. Transfer 1 ml of upper aqueous phase to a 2.0 ml microtube.
   For the matrices reported in RED in Table 1, it is suggested to split 3 ml of upper aqueous phase into 3 individual 2.0 ml microtubes.
   **Note: the upper phase is aqueous; if samples contain fats or oils the aqueous phase lies in the intermediate phase.**
6. Add 0.8 ml of Buffer E and mix well by vortexing for 1 minute, then centrifuge the sample at 10000 rpm for 5 minutes. The sample will appear parted in two liquid phases.
   **Note: the lower phase must be clear with no debris. If not, repeat the centrifugal step.**
7. Transfer the amount of lower aqueous phase indicated in Table 1 to a polypropylene tube containing 5 ml of Buffer T.
   **Note: avoid picking the proteins located in the intermediate phase. We suggest first to discard the supernatant and then pipette the subnatant avoiding the intermediate phase.**
   For matrices reported in RED in Table 1, collect the aliquots of subnatant previously split and transfer into a tube containing 7.5 ml of Buffer T.
8. Mix gently by inversion to avoid DNA shearing stress.
   **Note: Buffer T contains a pH indicator that turns from yellow to red when pH > 7.5. In case the colour turns to red, acidify the extract by adding glacial acetic acid (CH3COOH) dropwise until the colour of the buffer turns to yellow.**
   **Some extracts (e.g. those coming from gastronomic specialties) may contain pigments that permanently mask Buffer T colour in these cases add 80 µl of glacial acetic acid to guarantee the proper pH.**




9. When required by your matrix (see Table 1) filter the solution through a cellulose acetate membrane filter (0.45 µm pore size) disk inserted into a 10 ml syringe; otherwise proceed directly to the next step.
10. Connect DNA binding columns to the vacuum box as indicated in Figure 1.
    A. Purification column supplied with ION Force kit.
    B. Syringe 10 ml Luer-lock reservoir (ACC2911)
    C. Vacuum Box (EXD010-P)
    D. Stopcocks (EXD010-R)
11. Set the vacuum pump to pressure between -0.5 and -0.9 atm (alternatively, verify that the flow rate is not faster than 1 drop/second).
12. Pour the content of every tube in the respective column and wait the sample to flow completely through the column.
13. Wash the column by adding three times 0.75 ml of Buffer P (previously reconstituted with ethanol).
14. Remove DNA binding columns from the vacuum box; place each one in a collection tube and centrifuge at 7000 rpm for 5 minutes to drain completely the Buffer P (this step removes all ethanol traces which could inhibit the subsequent PCR reaction).
    **Note: in order to avoid samples cross-contaminations, after each use it is important to clean up stopcocks (Figure 1) soaking 1 hour in 5% bleach (sodium hypochlorite) followed by rinsing with distilled water.**
15. Discard the collection tube and transfer each column into a clean DNase/RNase free 1.5 ml microtube. Add 150 µl of Solution D (100 µl for low DNA content matrices and Generon "SpyX" products).
16. Wait 2 minutes, and then collect the purified DNA by spinning the columns 30 seconds at 500 rpm and continues at 14000 rpm for 5 minutes.
17. This DNA solution is stable one week at +4°C or 12 months at -20°C

# Workflow

1 Weigh sample and add Solution A

Solution A EXD003

20 ml

Shake briefly

2 Incubation 85°C per 1h

Spin 10 min the 50ml tube at 6000 < rpm < 10000

3 Transfer 1 ml of upper aqueous phase to a 2 ml tube and add 0,8 ml of Buffer E

Buffer E EXD013E

Vortex and spin 5 min at 10000 rpm

4 Transfer the sub-natant (lower aqueous phase) in a 15 ml tube filled with 5 ml of Buffer T

Buffer T EXD005

Assemble DNA binding column and syringe into the vacuum box

Buffer P EXD006

5 Wash 3 times with the Buffer P

Spin 5 min at 7000 rpm to drain wash solution

Solution D EXD004

6 Add 150 ul of solution D to elute DNA

7 Downstream Applications

**Table 1**: Examples and the amount of extracted material to be processed. **Note**: the suggested amount to be weighed for each matrix is not strictly binding and can be modified when keeping the ratio weight/volume of Solution A added in the lysis step.

| Matrix | Weight (± 0.2 g) | Aliquot of subnatant | Filtration |
|---|---|---|---|
| Animal feed, hay and tobacco | 2.5 g | 0.5 ml | Yes |
| Baby food | 5.0 g | 2.5 ml | Yes |
| Cheese | 2.5 g | 1.0 ml | Yes |
| Chips, snacks and biscuits | 5.0 g | 2.5 ml | Yes |
| Chocolate creams, coffee | 5.0 g | 1.0 ml | Yes |
| Raw Meat, fish and byproducts | 5.0 g | 0.5 ml | No |
| Corn flakes and honey | 5.0 g | 1 ml | No |
| Crackers, "grissini", bran, polenta | 5.0 g | 0.5 ml | No |
| Creams, yoghurt | 5.0 g | 2.5 ml | No |
| Flavours | 5.0 g | 2.5 ml | Yes |
| Flour and corn seeds, | 5.0 g | 0.5 ml | No |
| Flour and soy seeds | 0.5 g | 1.0 ml | Yes |
| Flours and feed formulations | 5.0 g | 0.5 ml | Yes |
| Food Additives (*): Acidity regulators, anticaking, antifoaming, emulsifiers, flavor enhancer, preservatives, humectants, coloring, … | 2.5 – 5 g (*) | 2.5 ml | No |
| Food Additives (*): Thickening agent and gelling agents (Agar, Pectin, Gelatin, Carrageenan, Guar gums, Xanthan, Acacia gum, …) | < 1.0 g (*) | 2.5 ml | Yes |
| Gastronomic specialties, Bakery and Ice creams | 5.0 g | 1.0 ml | Yes |
| Generon “SpyX” products | 5.0 g | 0.5 ml | No |
| Glucose syrup and fruit juice | 5.0 g | 2.5 ml | No |
| Gluten and sweet corn | 2.5 g | 1.0 ml | No |
| Jam | 5.0 g | 1.0 ml | Yes |
| Lecithin granular * | 10.0 g | 2.5 ml | No |
| Linseed | 1.0 g | 0.5 ml | Yes |
| Lyophilized products and cocoa powder | 2.5 g | 1.0 ml | Yes |
| Margarine and butter | 5.0 g | 2.5 ml | Yes |
| Milk powder, Serum powder | 2,5 g | 2.5 ml** | No |
| Modified starch | 2.5 g | 2.5 ml | No |
| Nougat and dried fruit | 5.0 g | 1.0 ml | Yes |
| Pasta | 2.0 g | 0.5 ml | No |
| Pet food, yeast and ferments | 5.0 g | 0.5 ml | No |
| Pizza | 5.0 g | 1.0 ml | Yes |
| Products soy based | 5.0 g | 1.0 ml | Yes |
| Pudding, mustard, jelly | 5.0 g | 2.5 ml | Yes |
| Raw and refined oils | 20.0 g | 2.5 ml | No |
| Ready-to-use cake mixes | 5.0 g | 2.5 ml | Yes |
| Rice and derivatives, cereals | 5.0 g | 1.0 ml | Yes |
| Sauces and dips | 5.0 g | 2.5 ml | Yes |
| Soy, blood and meat flour proteins | 2.5 g | 1.0 ml | Yes |
| Spices and dried vegetables | 2.5 g | 2.5 ml | Yes |
| Starches and sugars | 5.0 g | 2.5 ml | No |
| Sugar Alcohols | 5.0 g | 2.5 ml | No |
| Sweets, candy fruit | 5.0 g | 2.5 ml | Yes |
| Tomato concentrate | 5.0 g | 2.5 ml | Yes |
| Various seeds (canola, buckwheat, barley…) | 2.5 g | 0.5 ml | Yes |
| Vegetable matrices | 2.5 g | 0.5 ml | No |
| Vitamin and mineral supplements | 5.0 g | 0.5 ml | Yes |
| Wheat flour | 2.0 g | 0.5 ml | Yes |

(*) given the diversity of Food Additive group, in this case we suggest an in-house evaluation of the most proper sample weight based on the texture and characteristics of the unknown sample to optimize its manipulation during DNA extraction phase.
(**) Elute with 100 ul of Solution D.

## 6 – Specific DNA extraction from particular matrices

1. Transfer into a 15 or 50 ml tube the requested quantity as indicated in the table below.

| Matrix | Weight (± 0.2 g o ml) | Solution A | Incubation time | Aqueous phase to be transferred | Buffer E | Aliquot of subnatant | Buffer T |
|---|---|---|---|---|---|---|---|
| Animal Milk or Serum | 5 ml | 20 ml | 60 min | 3 ml | 3 ml | 3 ml | 7.5 ml |
| Enrichment media | 1 ml | 9 ml | 15 min | 1 ml | 0.8 ml | 0.9 ml | 5 ml |
| Filters, swabs | 1 unit | 1.5 ml | 15 min | Not necessary | Not necessary | 1 ml | 5 ml |
| Wine and beverages | 8 ml | 2 ml | 30 min | 4.5 ml | 1.5 ml | 3 ml | 5 ml |
| Feathers and plumes | 2 units | 1 ml | 60 min | 1 ml | 0.8 ml | 0.5 ml | 5 ml |
| Cooked meat | 5 g | 20 ml | 30 min | 1 ml | 0.8 ml | 0.5 ml | 5 ml |
| Blood | 1 ml | 9 ml | 15 min | 1 ml | 0.8 ml | 0.9 ml | 5 ml |

**For these matrices the filtration through a cellulose acetate membrane filter is not necessary.**

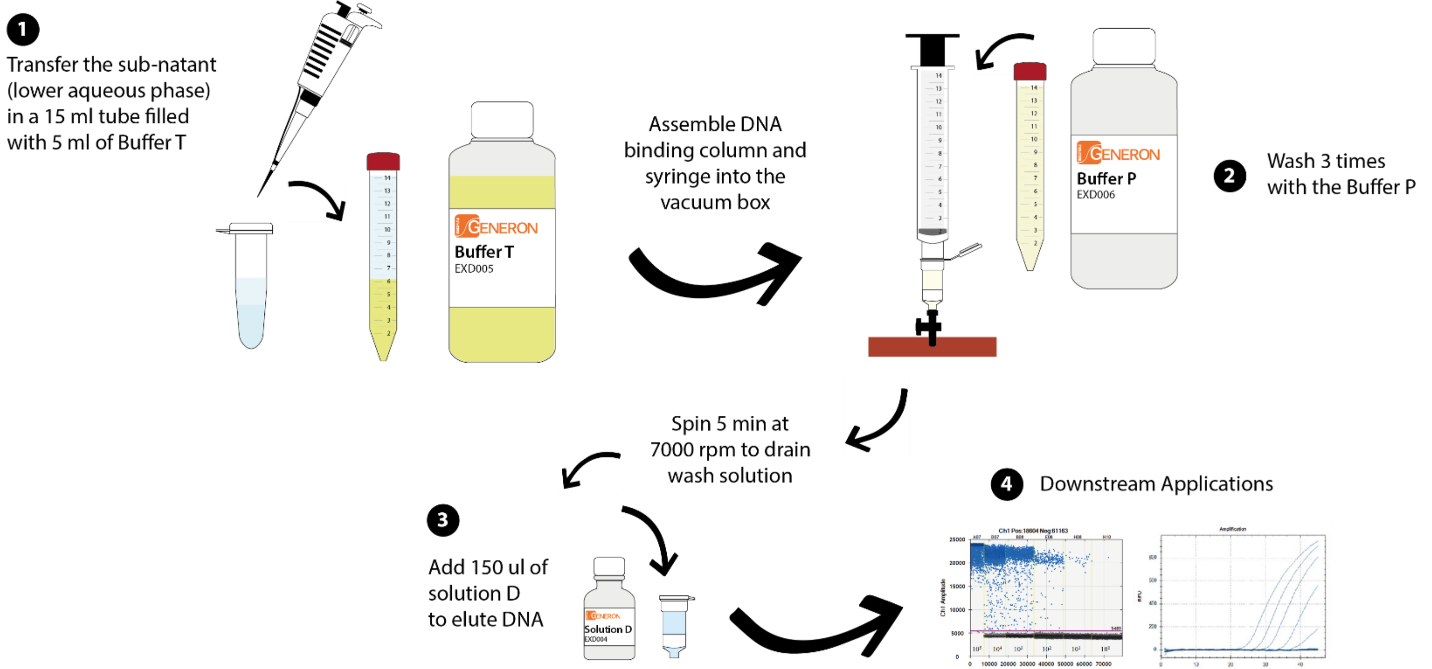
**Animal Milk:** centrifuge between 6000 and 10000 rpm for 20 minutes and remove the supernatant made of serum and fats, leave the pellet only.

**Filters**: using a vacuum or positive pressure source, filter the entire liquid sample through a membrane using aseptic techniques.

**Swab**: using a sterile cotton swab, intensively swab surface (maximum area of 100 $cm^2$) under test.

**Feathers and plumes**: cut two feathers into 2-3 parts (1.5 cm beginning from the bulb).

2. Add the amount of **Solution A** reported in the table above and then shake to homogenize the content.
3. Incubate at **85°C ± 2°C** following the time indications in the table above, shake 2-3 times during the incubation period.
4. Centrifuge the sample at speed between **6000 and 10000 rpm** for 10 minutes.
5. Transfer the amount of aqueous phase indicated in the table above to a 2 ml or to a 15 ml tube (on the basis of your sample matrix). **Note: the upper phase is aqueous but in fatty/oil samples the aqueous phase lies in the intermediate phase.**
6. Add the amount of Buffer E indicated in the table above and shake vigorously 1 minute, then centrifuge the sample at 10000 rpm for 5 minutes. **Note: the subnatant must be clear. If not, repeat the centrifugal step**.
7. Transfer the amount of subnatant indicated in the table above (avoid to pick up the proteins located in the intermediate phase) and then proceed as depicted in the figure below:



# 7 –DNA extraction from fluid lecithins

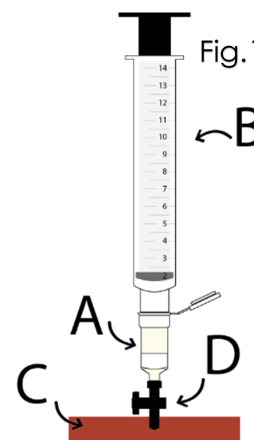
Warm up your fluid lecithin sample at 40°C at least for 20 min before starting the extraction.

1. Transfer into a 50 ml tube the requested quantity indicated in the table below.

| Matrix | Weight (± 0.2 g o ml) | Solution A | Incubation time | Aqueous phase to be transferred | Buffer E | Aliquot of subnatant | Buffer T |
|---|---|---|---|---|---|---|---|
| Fluid Lecithin | 2 g | 5 ml | 30 min | All (~ 3.5 ml) | 3 ml | All (~ 2.7-3.2 ml) | 15 ml |

Add the amount of **Solution A** reported in the table above and thoroughly vortex the sample for about 1 minute to homogenize the content (if 1 minute is not sufficient, continue vortexing until the mix is fully homogenized).

2. Incubate at **85°C ± 2°C** following the time indications in the table above. A thermomixer with speed set at 800 rpm is recommended.
3. Centrifuge the sample at **6000 rpm** for 10 minutes.
4. Transfer the amount of aqueous phase (all the lower phase) indicated in the table above to a **50 ml tube** using a pipette. If some particles are transferred they will be removed in the next step.
5. Add the amount of Buffer E indicated in the table above and vortex or shake vigorously 1 minute, then centrifuge the sample at 10000 rpm for 10 minutes. **Note: the subnatant must be clear (i.e. free of solid particles). If not, repeat the centrifugal step.**
6. Transfer all the subnatant (in the table above is indicated an average value) in 15 ml of Buffet T. Mix gently by inversion. (The user may use a 50 ml tube or alternatively split the Buffer T in two 15 ml tubes: one tube with 10 ml Buffer T + 2 ml aliquot of subnatant **and** a second tube with 5 ml Buffer T + the remaining aliquot of subnatant).
7. **Note: Buffer T contains a pH indicator that turns from yellow to red when pH > 7.5. In case the colour turns to red, acidify the extract by glacial acetic acid (CH3COOH) drop by drop to correct the pH value until the colour of the buffer turns to yellow. Some extracts (e.g. those coming from gastronomic specialties) may contain pigments that permanently mask Buffer T colour change, in these cases 80 µl of glacial acetic acid are enough to guarantee the turning oh pH.**
8. Connect DNA binding columns for DNA transfer to the vacuum box as indicated in Figure 1.
   A. Purification column supplied with ION Force kit.
   B. Syringe 10 ml Luer-lock reservoir (ACC2911)
   C. Vacuum Box (EXD010-P)
   D. Stopcocks (EXD010-R)




9. Set the vacuum pump to pressure between -0.5 and -0.9 atm (alternatively, verify that the flow rate is not faster than 1 drop/second).
10. Pour the content of every tube in the respective column and wait the sample to flow completely through the column.
11. Wash the column by adding three times 0.75 ml of Buffer P (previously reconstituted with ethanol).
12. Remove DNA binding columns from the vacuum box; place each one in a collection tube and centrifuge at 7000 rpm for 5 minutes to drain completely the Buffer P (this step removes all ethanol traces which could inhibit the subsequent PCR reaction). **Note: in order to avoid samples cross-contaminations, after each use it is important to clean up stopcocks (Figure 1) soaking 1 hour in 5% bleach (sodium hypochlorite) followed by rinsing with distilled water.**
13. Discard the collection tube and transfer each column into a clean DNase/RNase free 1.5 ml microtube. Add 100 µl of Sol. D
14. Wait 2 minutes, and then collect the purified DNA by spinning the columns 30 seconds at 500 rpm and continues at 14000 rpm for 5 minutes.
15. This DNA solution is stable one week at +4°C or 12 months at -20°C

## 8 – Results

Different samples have been extracted with ION Force DNA Extractor Fast. Categories range from raw materials such as soy, meat or fresh vegetables, to semi-finished materials like lecithin and to processed foods such as baby food, snacks or dietary supplements.

The table below depicts the product types and the DNA yield after extraction of 1 gram. Data represent an averaged value obtained from similar matrices produced by different manufacturers (n = 10 for each sample kind).

| Matrices | ng in 150 µl | Matrices | ng in 150 µl |
|---|---|---|---|
| Animal feed | 2124 | Instant mashed potatoes | 186 |
| Baby food | 1314 | Lecithin (fluid soy) | 17-90 (in 100 µl) |
| Bakery | 1551 | Lecithin (soy powder) | 710 |
| Barley | 2070 | Margarine and butter | 174 |
| Beverages | 152 | Meat | 3623 |
| Buckwheat | 1034 | Milk powder | 737 |
| Candies and jams | 176 | Modified starch | 257 |
| Canola | 520,5 | Oil (corn) | 50 |
| Corn flour | 4521 | Oil (olive) | 35 |
| Chocolate | 140 | Oil (soy) | 65 |
| Cocoa powder | 342 | Pasta | 1988 |
| Corn | 2651 | Pet food | 1856 |
| Corn flakes | 1889 | Pizza | 636 |
| Corn grits | 1451 | Pudding and jelly | 135 |
| Corn proteins | 2373 | Rice | 2258 |
| Cornmeal mush | 1244 | Sauces and dips | 3803 |
| Cotton seeds | 717 | Soy | 1356 |
| Creams | 348 | Soy (proteins) | 3125 |
| Cured meats | 2930 | Soy (toasted) | 323 |
| Dried fruit | 720 | Spices | 4453 |
| Fish | 2399 | Starch | 381 |
| Flax seeds | 320 | Sweet and savory snacks | 324 |
| Flour | 696 | Tobacco | 8349 |
| Fresh vegetables | 3392 | Tomato concentrate | 3323 |
| Gastronomic specialities | 3051 | Vitamin and mineral supplements | 41 |
| Glucose syrup | 27 | Wheat | 8123 |
| Hay | 3839 | Whole wheat flour | 809 |
| Honey | 41 | Yeast | 639 |
| Ice creams | 162 | Yoghurt with fruit | 207 |

## 9 – Disclaimers

Generon S.p.A. guarantees the buyer exclusively concerning the quality of reagents and of the components used to manufacture the product. Generon S.p.A. is not responsible and cannot anyway be considered responsible or jointly responsible for any possible damages resulting from the utilization of the product by the user and from the data obtained.

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