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Grains DNA extraction kit
MBK0064 50 extractions

INTENDED USE

Grains DNA extraction kit allows the isolation of genomic DNA from corn, flour and derivative products such as pasta.

INTRODUCTION

The composition in cereals of specific foods is a key factor for the quality of the final product. The cereal of choice for the production of pasta is durum wheat due to its special features such as colour and protein content, that affect the quality of pasta. *Grains DNA extraction kit* is formulated for the DNA isolation from pasta and flours in order to verify, through the use of Grain quantitative kit (Diatheva, MBK0062), the content of soft wheat in the final product caused by cross contaminations that may occur during growing, harvesting and handling practices of durum wheat. The production and sale of pasta is strictly regulated by the current Italian law: only a maximum of 3% soft wheat can be tolerated in dry pasta.

PRODUCT DESCRIPTION

The *Grains DNA extraction kit* permits to simplify the DNA isolation process and to obtain a purified DNA suitable for amplification in Real-Time PCR.
The *Grains DNA Extraction Kit* combines an efficient lysis process with a purification system, using a binding buffers in combination with columns. The silica membrane was optimized for an high yield of DNA and low affinity for the binding of impurities.
After homogenization of the sample, is added the GL Lysis buffer containing chaotropic salts, denaturing agents and detergents that ensure optimal lysis action on the sample which is then clarified by centrifugation. The add of GB Binding buffer creates the optimal conditions for the binding of DNA to the silica membrane of the column. The impurities are removed with a simple washing, and the purified DNA was eluted in a low ionic strength buffer.

In Table 1 is shown a list of samples that has been tested to verify the functionality of kit.

Table 1 Foods tested to verify the functionality of Grains extraction kit

Food	Sample
Corn	Oat
	Colza
	Spelt
	Sunflower
	Buckwheat groats
	Flax
	Millet
	Barley
	Rye
	Sesame
	Soya
Flour	Soft wheat
	Durum wheat
	Maize flour
	Soya meal
Derivative product	Mixed cereals
	Oat bran
	Breadsticks
	Dry pelleted feed
	Sesame bread
	Bread crumbs
	Soya expeller
	Pasta

KIT CONTENTS

Component	Description	Volume or Unit
GL	Lysis buffer	50 ml
GB	Binding buffer	60 ml
GW	Washing buffer	27 ml
GE	Elution buffer	14 ml
	Lysis tubes 2 ml	50 unit
	Columns	50 unit

STORAGE

Store at room temperature (15-25 °C) and in the dark. Do not use after the expiry date.

PRECAUTIONS

- The test should be performed only by qualified and authorized personnel;
- Do not use reagents after the expiry date;
- The degradation of the reagents could cause wrong results;
- **GL Lysis buffer contains an insoluble compound that settles to the bottom, shake it before use**;
- **GL Lysis buffer** and **GB Binding buffer** contain irritant and inflammable salts for eyes and skin. Avoid the ingestion. Is recommended the use of gloves and goggles.

ADDITIONAL EQUIPMENT

- Equipment necessary for the homogenization of sample;
- Pipettes and tips with filter;
- 1,5 ml and 2 ml microcentrifuge tubes;
- Microcentrifuge;
- Vortex;
- Ethanol (96-100%);
- Water bath or heating block set at 75 °C.

PROCEDURE

Before start:

- Reconstitute **GW-Washing buffer**, add **33 ml** of 96-100% ethanol and mix well. Label the bottle after ethanol is added.
- Warm water bath or heating block at 75°C.

Procedure

1. Homogenization of sample

Homogenize the sample using a commercial system or mortar. A good homogenization is the basis for optimal system performance.

2. Lysis of sample

2.1 Transfer 200 mg (100 mg for soya and derivatives, colza and sunflower) of homogenized food sample in 2 ml microcentrifuge tube (provided).
2.2 Add 800 μl of **GL Lysis buffer**, taking care to mix immediately before the use in order to collect the mixture in suspension. Vortex for 20 seconds and incubate at **75 °C** for 20 minutes, possible with shaking.
2.3 Transfer the sample at -20 °C or in ice for 10 minutes.
2.4 Vortex briefly and centrifuge for 10 minutes at 12,000 x g at room temperature.

3. Binding to column

3.1 Transfer 200 μl of supernatant in a 2 ml microcentrifuge tube (not provided).
3.2 Add 1 ml of **GB Binding buffer** and mix gently by pipetting up and down.

NOTE: it is possible use a different volume of supernatant, but it is important that the ratio between GB Binding buffer and supernatant is:

supernatant volume : **GB** volume = 1 : 5

3.3 Transfer 600 μl of mixture in the columns (including any precipitate) and centrifuge for 1 minute at 12000 x g at room temperature. Discard the flow-through.
3.4 Apply the remaining mixture and repeat the step 3.3.

4. Washing bound DNA

4.1 Add 700 μl of **GW Washing buffer** (reconstituted, see "Before start") and centrifuge for 1 minute at 12,000 x g at room temperature. Discard the flow-through.
4.2 Centrifuge at 12,000 x g for 1 minute to remove residual washing buffer.

5. Elution of purified DNA

5.1 Insert the column into a clean 1,5 ml microcentrifuge tube (not provided) and add to the center of the column bed 70 μl of **GE Elution buffer** (*Optional:* for a better extraction yield, pre-warmed the GE elution buffer at 65 °C).
5.2 Incubate at room temperature for 5 minutes and centrifuge at 12,000 x g for 1 minute. The microcentrifuge tube contain the eluted DNA.

The purified genomic DNA can be stored at 2-8 °C for a few days. For longer term storage, -20 °C is recommended. Before reusing it always allows thawing and mixing well by gentle vortexing.

If using the *Grain quantitative kit* (Diatheva, MBK0062) for the quantification of

***Triticum aestivum* (soft wheat) in *Triticum* spp., it is recommended to dilute the DNA extract. Refer to the product information of the *Grain quantitative kit*.**

Quantification of DNA

To estimate approximatively the amount of the extracted DNA use electrophoresis on agarose gel (with appropriate reference standard) and/or the absorption measurement at 260 nm (A_{260}) in a spectrophotometer. To ensure that the spectrometer gives a value indicative of the quality/quantity of the extracted DNA, we recommend:
-the measurement at 260 – 280 nm;
-the calculation of 260/280 ratio to evaluate the purity of the DNA.

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