Data sheet

HigherPurity™ Food DNA Purification Kit

Cat. No: AN0240 (50 reactions)
Cat. No: AN0241 (100 reactions)

Description

This kit has been optimized for an efficient and fast purification of total DNA from food samples (raw material and processed food).
After the samples have been homogenized, the DNA can be extracted with the extraction buffer, lysis mixtures should be cleared by centrifugation or filtration in order to remove contaminants and residual cellular debris. The clear supernatant is then mixed with the binding buffer, proteinase K and isopropanol to create conditions for optimal binding to the silica membrane column. After washing with two different buffers for efficient removal of potential PCR inhibitors, DNA can be eluted in low salt buffer or water, and is ready-to-use in subsequent reactions.

Advantages/Features:

- Silica membrane technology.
- Rapid purification of high-quality, ready to use DNA.
- Even low amounts of partially degrade DNA can be purified from complex matrices.
- Complete removal of contaminants and inhibitors for reliable downstream applications.
- Sample size: up to 200mg.

Applications:

- DNA from complex matrices, processed food, soya, chocolate, cereals, meat, animal feed.
- Detection of genetically modified material in food products.
- DNA suitable for PCR, real-time PCR, Southern blotting, enzymatic reactions.
- Detection of specific DNA in animal feed.

Kit Components

	AN0240	AN0241
Minispin columns	50	100
Collection tubes (2 mL)	100	200
Lysis Solution A (LSA)	65 ml	130 ml
Lysis Solution B (LSB)	15 ml	30 ml
Proteinase K*	30 mg	60 mg
WB1 Buffer**	16.5 ml	33 ml
WB2 Buffer**	10 ml	20 ml
EB buffer	10 ml	20ml

*Dissolve Proteinase K in 1.3 ml nuclease-free water to obtain stock solution. The Proteinase K solution can be stored for several days at 2–8 °C. For longer-term storage, the unused portion of the solution may be stored in aliquots at –20 °C until needed. This product as supplied is stable at room temperature.

Add the volume ethanol (96%-100%) specified **[Not included] to WB1 and WB2 Buffer prior to initial use (see bottle label for volume). After ethanol has been added, mark the bottle to indicate that this step has been completed.

Kit Storage:

Store the kit at room temperature. If any kit reagent forms a precipitate, warm at 55–65 °C until the precipitate dissolves, and allow to cool to room temperature before use.

Please wear gloves when using this product. Avoid all skin contact with kit reagents. In case of contact, wash thoroughly with water.

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Assay procedure

1. Add 100-200 mg of sample into a 2 ml microtube [Not included].
2. Add 1.2 mL of **Lysis Solution A (LSA)** + 25 µl **Proteinase K** and mix well by vortexing.
3. Incubate the sample at 65 °C for 30 minutes. Votex the sample during the incubation.

The main and more important step to obtain good yield is a good homogenization of the sample that will be specific for each sample type. The lysis procedure is most effective when well homogenized, powdered samples are used. To achieve this, we recommend grinding with a pestle and mortar in the presence of liquid nitrogen or using steel beads.
Commercial homogenizers can also be used.
As general norm in solid samples (sausages, etc), to prepare several fragments and to homogenize with a hand electric homogenizer; In powdered solid samples (flours, etc.) to homogenize with a hand electric homogenizer; In solid samples of great size (corn flakes, chocolate, cookies, etc) to use a grinder of coffee to pulverize a big sample and then to weigh the required quantity of powder; In liquid samples to use 200 µl directly.

4. Centrifuge at 14 000 rpm for 5-10 minutes. A pellet will appear and in the surface a layer of fat, to introduce the pipette tip crossing this superficial layer of fat, only trying to pick up 500 µl of supernatant that it is the transparent liquid with color (to avoid to catch pellet and superficial layer) and to place in a 1.5 ml microtube [Not included].
5. Add 250 µl **Lysis Solution B (LSB)** to 500 µl of supernatant and mix well by vortexing.
6. Assemble a spin column with one of the provided collection tubes. Add sample mixture onto the spin column. Close the cap and centrifuge at 10 000 rpm for 1 minute. Discard the flow-through and reassemble the spin column to a new Collection Tube.
7. Carefully open the spin column and add 500 µl **Buffer WB1** (ethanol added). Close the cap and centrifuge at 12 000 rpm for 1 min. Place the spin column in a new 2 ml collection tube, and discard the flow-through.
8. Carefully open the spin column and add 700 µl **Buffer WB2** (ethanol added). Close the cap and centrifuge at 14 000 rpm for 1 min. Discard the the flow-through.
9. Centrifuge at full speed for an additional 2 min to dry the spin column and discard the collection tube containing the filtrate.

This step will avoid the residual liquid to inhibit subsequent enzymatic reactions.

10. Place the spin column into a new 1.5 mL microcentrifuge tube [Not included]. Carefully open the spin column and Add 50-200 µl of **Elution Buffer** or ddH_2O to the membrane centre. Close the cap and incubate for 2 min at room temperature, then centrifuge at full speed for 1 min to elute DNA.
11. The purified genomic DNA can be stored at 2-8°C for a few days. For longer term storage, -20°C is recommended.

For Research Use Only. Please refer to **www.canvaxbiotech.com** for Material Safety Data Sheet of the product.

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