

DNA Cleaning Columns

Kit for the purification of DNA,
e.g. from food, feed and grains

Cat. Nos: 5224700305 and 5224700310

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TECHNICAL SERVICE

If you have any questions or experience any difficulties regarding this kit or Eurofins GeneScan Technologies products in general, please do not hesitate to contact us. Eurofins GeneScan Technologies customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at Eurofins GeneScan Technologies. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call the GeneScan Technologies Technical Service Department or local distributors.

Eurofins GeneScan Technologies GmbH

Engesser Str. 4

79108 Freiburg, Germany

Phone: + 49-(0)761-5038-100

Fax: + 49-(0)761-5038-111

kits@eurofins.com

www.eurofins.com/kits

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Table of Contents:

1. COMPONENTS OF THE KIT	3
2. STORAGE AND PREPARATION OF THE KIT	3
3. INTRODUCTION	4
4. QUALITY CONTROL AND LIMITATIONS	5
5. GENERAL GUIDELINES	6
6. CENTRIFUGE INFORMATION	8
7. PROTOCOL	9
8. PRODUCT WARRANTIES AND SATISFACTION GUARANTEE	10
9. IMPORTANT NOTES	11
TECHNICAL SERVICE	12

1. Components of the Kit

The DNA Cleaning Columns kit contains 50 (for cat. no. 5224700305) or 100 (for cat. no. 5224700310) DNA cleaning columns with green caps, pre-packed with Sephacryl® resin and equilibrated in TE buffer (pH 7.6).

Important Note: This kit is not recommended for RNA purification, for removing excess adaptors from cDNA reactions or for removing unincorporated nucleotides from end-labelled oligonucleotides.

2. Storage and Preparation of the Kit



Store the kit at 4° C.

Before using a column, open the bottom closure.

Kits, their components and instructions for use are subject to alterations.

3. Introduction

This kit is designed for the purification of DNA for a wide range of applications. It yields DNA of high quality and purity. The test kit is ideal for pre- and post-PCR clean-up (e.g. before real-time PCR).

Spin-column chromatography offers many advantages over traditional liquid chromatography:

- **Speed:** Just spin, load sample, spin, and collect the purified product in less than 4 minutes.
- **Convenience:** No column packing, column is pre-packed and equilibrated
- **No sample dilution:** The pre-spin step removes most of the storage buffer so that when the sample is purified, it elutes in a volume equivalent to the applied sample volume.
- **High throughput:** Numerous samples can be processed simultaneously.
- **Capacity for a range of sample volumes and quantities of nucleic acid:** Good product yield with sample volumes from 10 – 100 µL. Nanogram to milligram quantities of nucleic acids can be purified with excellent results.

Gel filtration resins do not exhibit a fixed exclusion limit when used in a spin-column format. Exclusion limits of gel filtration resins are only meaningful in continuous flow processes where the molecules being purified have sufficient time to reach an equilibrium between the time spent in the pores of the gel filtration medium and the time spent in the eluent stream.

In spin-column chromatography, the observed exclusion properties that allow the product to pass through the gel while the smaller sample impurities are retained depends on experimental factors, such as: the depth of the resin bed, the resin used, sample volume, product size, and the g forces used in the purification process.

4. Quality Control and Limitations

The columns are tested for DNase and RNase.

The columns may cause positive PCR results for nptII analyses with DNA purified with the columns in rare cases, thus a confirmation of nptII-positive PCR results with further detection systems for the respective GMO or modification is recommended.

5. General Guidelines

20x Rule

The best results are obtained when the smallest product being purified is at least 20 times larger than the largest impurity. A difference in size smaller than 20-fold, may affect either purity or yield.

Nonspecific binding

The nonspecific binding of the columns is relatively insignificant, allowing purification of samples in the nanogram range.

Retention

For a given sample volume, product retention inversely correlates to molecular size. As the size of the product increases, its relative retention decreases.

Purity vs Yield

In general, purity is inversely proportional to yield. Larger sample volumes will provide higher yield but lower purity, and vice versa.

Load 10 – 100 µL onto a column for all applications.

For occasions in which your current sample volume falls outside the volumes mentioned above, we recommend the following sample adjustments:

For **large** sample volumes, choose one of two options :

- 1) Apply to the column an aliquot of the sample no larger than the recommended loading volume for the application. Use a number of columns correlating to your total volume to purify the entire sample.
- 2) Precipitate the entire sample and redissolve it in an appropriate volume.

For **small** sample volumes, dilute the sample to a larger volume to increase product recovery.

6. Centrifuge information

Before using a column, it is important to calculate the appropriate speed at which the column should be centrifuged. In order to do so, use the following formula:

$$RCF = (1.12) \times (r) \times (rpm/1000)^2$$

where

RCF = relative centrifugal force; r = radius in mm measured from center of spindle to bottom of rotor bucket; and rpm = revolutions per minute.

For a force of 800 x g, the above equation resolves to:
rpm = (1000)($\sqrt{714/r}$)

For example, with a microcentrifuge rotor having a radius of 110 mm, the appropriate speed would be 2548 rpm. If you do not have a variable-speed microcentrifuge, you may use a clinical centrifuge set at a speed corresponding to 800 x g. You may also use a single-speed microcentrifuge, which may result in a small loss of column performance.

For Heraeus Biofuge 13 or Eppendorf 5415C with an 18-position fixed-angle rotor: use 3000 rpm.

7. Protocol

The procedure below is based on use with a variable-speed microcentrifuge. Two sample tubes (1.5 ml) per sample are needed.

- 1) Resuspend the resin in the column by vortexing.
- 2) Open the bottom plug and loosen the cap one-fourth turn. Place the column in a 1.5 ml screw-cap microcentrifuge tube for support. Alternatively, cut the cap from a flip-top tube and use this tube as a support.
- 3) Pre-spin the column at 800 x g in a microcentrifuge with a fixed-angle rotor for 1 min.
- 4) Place the column in a new 1.5 ml tube, remove and discard the cap and slowly apply the sample (10 – 100 µL) to the top-center of the resin, being careful not to disturb the bed.
- 5) Spin the column at 800 x g for 2 min. The purified sample is collected in the bottom of the support tube.

8. Product Warranties and Satisfaction Guarantee

Eurofins GeneScan Technologies GmbH ("GeneScan") warrants the products manufactured and/or sold by it will be free of defects in materials and workmanship when used in accordance with the applicable instructions for a period equal to or shorter of one year from the date of shipment of the product(s) or the expiration date marked on the product packaging under the storage conditions, recommended in the instructions and/or on the package. Application protocols published by GeneScan are intended to be only guidelines for the buyers of the products. Each buyer is expected to validate the applicability of each application protocol to his individual application. GeneScan makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose. GeneScan's sole obligation with the respect to the foregoing warranties shall be, at its option, to either replace or to refund the purchase price of the product(s) or part thereof that proves defective in materials or workmanship within the warranty period, provided the customer notifies GeneScan promptly of any such defect. GeneScan shall not be liable for any direct, indirect or consequential damages resulting from economic loss or

property damages sustained by buyer or any customer from the use of the product(s). A copy of Eurofins GeneScan Technologies GmbH terms and conditions can be obtained on request, and is also provided with our product/price lists.

9. IMPORTANT NOTES

Warning: This kit is for research use only. Not intended or recommended for diagnostic purposes. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet and/or safety statement for specific advice.