NICK Columns

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- For rapid purification of labeled DNA (≥ 20 bases in length) from unincorporated radiolabeled nucleotides using gravity-flow chromatography
- Prepacked with Sephadex G-50 DNA Grade (*see* Sephadex G-50 DNA Grade (/shop/molecular-biology/purification/gel-filtration-columns/illustra-sephadex-g-50-dna-grade-p-00113) and Sephadex G-100 DNA Grade (/shop/molecular-biology

/purification/gel-filtration-columns/illustra-sephadex-g-100-dna-grade-p-00198)) in distilled water with 0.15% Kathon CG/ICP Biocide
- Can accommodate sample volumes up to 100 µl

NICK columns are ready for immediate use and operate by gravity flow. Tests show that at least 97% of radioactivity applied in a nick-translated mixture elutes in two well-separated peaks, corresponding to labeled DNA molecules and unincorporated nucleotides, respectively. At least 90% of the applied DNA is recovered and can then be used in DNA hybridization protocols. Maximum sample volume is 100 µL.

Nick translation is a technique in which DNA Polymerase I is used to replace some of the nucleotides of a DNA sequence with their labeled analogues, creating a tagged DNA sequence which can be used as a probe in fluorescent in situ hybridization (FISH) or blotting techniques. It can also be used for radioactive labeling in techniques such as Southern blotting.

This process is called nick translation because the DNA to be labelled is treated with DNase to produce single-stranded "nicks." This is followed by replacement in nicked sites by DNA polymerase I, which elongates the 3' end, removing nucleotides by 5'-3' exonuclease activity, and replacing them with dNTPs. To label a DNA fragment for use as a probe in blotting one of the incorporated nucleotides provided in the reaction is radiolabeled, or a fluorophore can be attached instead for fluorescent labelling, or is used as an antigen for immunodetection.

Sephadex G-50 DNA Grade can be used in a wide range of applications, including desalting DNA, buffer exchange, and removal of unincorporated nucleotides from end-labelled oligonucleotides. Sephadex G-50 can be packed into empty MicroSpin columns for use in these applications.

Sephadex G-50 is one of five different G-types ranging from G-10 for small molecules to G-75 for larger molecules. Sephadex G-50 is a well-established gel filtration resin for desalting and buffer exchange of biomolecules > 30 000 molecular weight, and with a spin protocol can be used for DNA and oligo purification of molecules greater than 20 bases in length. Alternatively, Sephadex G-25 can be used. Sephadex G-25 has an exclusion limit of approximately Mr 5000, and with a spin protocol this means it can be used for any DNA greater than 10 bases in length.

Both types of Sephadex are therefore highly suitable for the purification of oligonucleotides or very small DNA fragments following synthesis or in radioactive labeling.

Sephadex G-25 DNA Grade (/shop/molecular-biology/purification/gel-filtration-columns/illustra-sephadex-g-25-dna-grade-p-00054) and Sephadex G-50 DNA Grade (/shop/molecular-biology/purification/gel-filtration-columns/illustra-sephadex-g-50-dna-grade-p-00113) are sold separately.

Product Specifications

Amersham NICK Columns

Parameter	Amersham NICK Columns
Bed dimensions	9 × 20 mm
Bed height	20 mm
Binding Capacity/Column	100 µg
Column i.d.	9 mm
Sample volume	< 100 µl
Storage	4 to 30°C
Pack size	20 columns