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Application Note

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Rapid DNA Removal from Mammalian Cell Lysates By Ion Exchange Purification with Vivapure®

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Introduction

Routine preparation of cell lysates for western blotting involves pre-treatment of the sample to prevent the DNA present from interfering with the electrophoresis on SDS polyacrylamide gels. This procedure may involve shearing the DNA by passing the sample through a small gauge needle or by sonication.

This is a time consuming process which is difficult to repeat consistently and in many cases does not resolve the problem, with samples remaining too viscous to enter the gel efficiently. On the other hand, treatment with nucleases such as DNase contaminates the sample with additional proteins. Furthermore, the incubation time required poses a risk for degradation of the target molecule. Vivapure® ion exchange spin columns offer a solution to this problem; within a few minutes DNA can be effectively removed from a lysate without the loss or degradation of proteins and the samples are then ready for subsequent analysis by SDS PAGE.

Materials and Methods

Monolayers of an astrocyte cell line, A7[1], were grown to confluence, washed with PBS and then harvested by scraping into RIPA buffer (1x PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, 0.1% SDS) to which a protease inhibitor cocktail (Sigma) had been freshly added.

The protein lysate was passed through a Vivapure® D Mini anion exchange spin column, pre-equilibrated with 100 mM Tris/HCl, pH 7.5, 0.5 M KCl. 300 mg of lysate was brought up to 1.5 mL with the same buffer and added to the column in 3 x 500 µL aliquots, spinning each time for 2 mins at 13,000 rpm in a micro-centrifuge. The column was then washed twice with 400 mL of 100 mM Tris-HCl (pH 7.5), 500 mM KCl. All eluents were collected and pooled.

The pooled eluent sample was concentrated using a Vivaspin® 2 (10 kDa MWCO PES) and 10 mg of protein from the un-purified lysate or the concentrated post-column eluent were electrophoresed on a 1% TBE agarose gel containing ethidium bromide. DNA was visualized under UV light. A similar amount of both samples was also analyzed by protein gel electrophoresis, using a NuPAGE™ 4 to 12% Bis-Tris gel (Thermo Fisher Scientific) and staining of the proteins by SimplyBlue™ SafeStain.

Results

DNA was removed from the astrocyte cell lysate by ion exchange chromatography using Vivapure® D Mini centrifugal columns (Figure 1).

There was no loss or degradation of the astrocyte cell proteins following DNA removal by Vivapure® D and concentration with Vivaspin® 2 (Figure 2).

Figure 1.

TBE agarose gel of an astrocyte lysate before (1) and after (2) removal of DNA using Vivapure® D Mini anion exchange spin column. The DNA present in the lysate appears as a smear, indicating the presence of a range of different lengths when compared to the standard DNA marker (3).

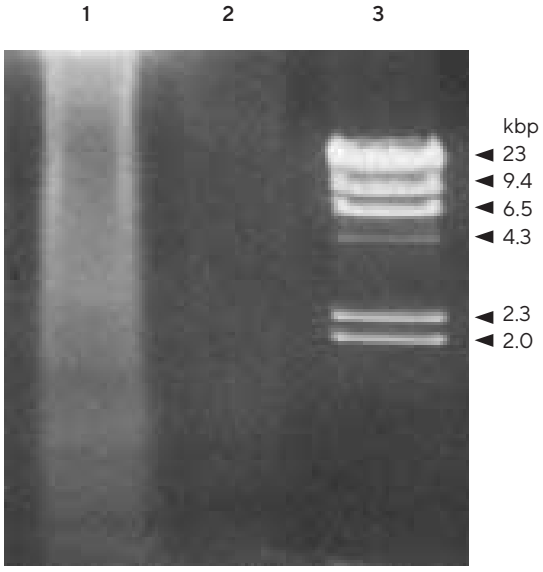
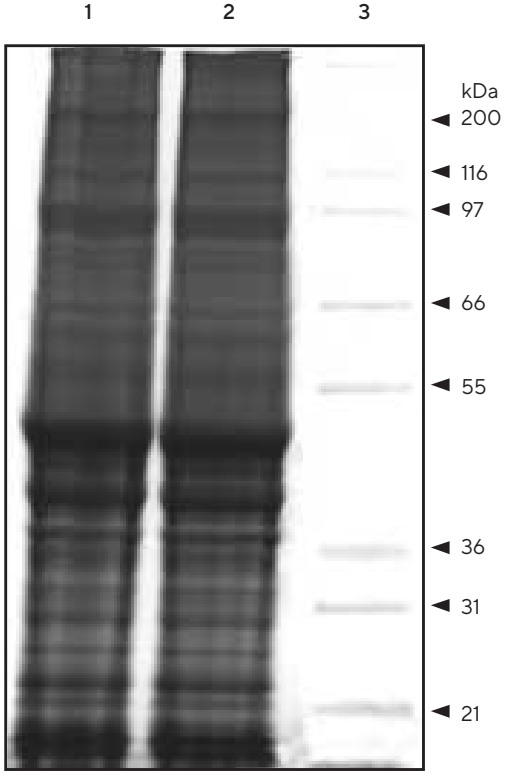


Figure 2:

NuPAGE™ 4 to 12% Bis-Tris gel of astrocyte lysate before (1) and after (2) removal of DNA by ion exchange with Vivapure® D Mini.



Conclusions

Vivapure® centrifugal ion exchange devices offer a very rapid and efficient alternative to conventional methods of removing DNA from cellular lysates prior to analysis or further purification of proteins. The cost of using expensive nucleases to digest DNA into small fragments may be avoided by using these spin columns for DNA removal. Furthermore, this method provides much greater efficiency of removing DNA from cell lysates than that obtained by mechanical shearing techniques with needles or sonication.

References

1. Geller, H.M. and Dubois-Dalcq, M. (1988) Antigenic and functional characterisation of a rat central nervous system-derived cell line immortalised by a retroviral vector, Journal of Cell Biology **107**, 1977-1986

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