

HI-FLOW[®] – NOVEL LARGE VOLUME COLUMNS FOR DNA EXTRACTION

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The ideal DNA isolation protocol should provide a maximum yield of DNA free from inhibitory compounds that can affect downstream applications. It also is important to minimize sample handling steps, as every manipulation provides an opportunity for contamination. Reduced sample manipulation also limits the loss of DNA through repeated extractions and transfers. The latter benefit becomes even more critical in cases where the quantity of sample (e.g., bone) available for DNA isolation is minimal.

Larger volumes of digestion buffer are being used to more fully demineralize pulverized bone samples. One way to handle the increased volume of crude extract is to employ ultrafiltration for sample concentration, buffer exchange, and removal of contaminants smaller than the nominal molecular weight cutoff of the device's filtration membrane. These devices must be used in conjunction with another purification method such as organic extraction or silica column purification in order to sufficiently reduce co-purifying inhibitory compounds. Silica based columns, slurries, and resins have long been available for DNA isolation, but the current methodology is geared toward extraction of DNA from small volumes. Limited volume has proven to be a substantial disadvantage of applying silica-based extraction methods to bone-derived samples. This study describes a silica-based device that is large enough to process the entire volume of crude extract in a single step, which greatly reduces time and manipulation necessary to perform purification. Silica columns (Hi-Flow columns) with a larger volume capacity have been produced (Generon Ltd, Berkshire, UK) to effectively handle commonly encountered working volumes of crude DNA extract. This simplification of the protocol substantially reduces the number of handling steps and sample transfers required, as well as eliminates the use of hazardous compounds such as phenol and chloroform.