

NOVEL LYSIS CHEMISTRY FOR EXTRACTION OF DNA FROM CALCIFIED TISSUES AND OTHER COMPLEX SAMPLE TYPES

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We report here on a novel lysis reagent, BTA (for Bone, Tooth and Adhesive) lysis buffer, that significantly enhances the yield and purity of DNA extracted from complex sample types such as calcified tissues and those with adhesive-containing substrates. Protocols optimized for this reagent integrate the effective lysis capability of BTA lysis buffer with existing PrepFiler extraction chemistry and workflows, facilitating streamlined high quality DNA preparation from these sample types.

Comparative chemistry studies on DNA extraction and genotyping were conducted with BTA on a set of standardized mock forensic samples including bone, tooth, E-cell-spotted adhesive tape, envelope flaps and cigarette butts. Endpoints for performance evaluation included: DNA yield and STR profile quality as determined by Intra-Color Balance (ICB) ratios; percentage of total alleles recovered; and number of informative loci returned. Overall in this study, BTA returned results that were either comparable or superior to other methods tested, which included Phenol/Chloroform/Isoamyl Alcohol (PCI) and commercially available kits. Most significantly, when DNA extractions were conducted on a particularly challenging, low DNA-content bone specimen, BTA's performance was superior to that of PCI, with respect to percent alleles recovered and number of informative loci returned from isolated DNA with Identifiler® and MiniFiler® PCR amplification kits. On this and another bone specimen, DNA yields with BTA and PCI were superior to those of other competitive chemistries tested; however, the DNA isolated from bone in BTA extractions was relatively free of inhibitors compared to that isolated in PCI extractions, as evidenced by internal positive control C_T values in downstream qPCR assays and by quality of STR profiles returned.

Beyond performance criteria alone, the BTA extraction process advances the field in a number of important ways. In comparison to PCI methods, the BTA method: (a) combines de-calcification, lysis and DNA extraction into a highly streamlined, integrated workflow (significantly reducing the likelihood of error and difficulty involved in DNA extraction); and (b) eliminates the need to use and dispose of potentially hazardous organic reagents.