

**ISOCODE® ID™, A DNA COLLECTION AND ISOLATION DEVICE FOR USE
WITH CLEAR BIOLOGICAL SAMPLES.**

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The collection of clear biological samples using paper-based devices, such as IsoCode®, has been beset with the issue of trying to determine where on the device the sample was placed. Identification of the exact sample application area is essential in order to obtain a punch for use with genetic typing procedures. We have developed a new form of IsoCode®, IsoCode® ID™ Sample Registration Matrix, designed to make sample registration of clear, oral samples easier. IsoCode® ID™ incorporates a purple color indicator that turns white in the exact area where the sample has been applied and dried. IsoCode® ID™, like IsoCode®, is a chemically modified 903™ paper that protects DNA contained in biological samples from degradation and microbial contamination. When a sample is applied to IsoCode® ID™, the chemistry lyses the cells, dissociates proteins from nucleic acids and destroys nucleolytic enzymes. As the sample dries, inhibitors of PCR are fixed to the paper matrix. DNA, suitable for PCR amplification and genetic typing, is eluted from the matrix in water by a simple heat step.

In this study, we show that IsoCode® ID™ is functionally equivalent to IsoCode® as judged by β-globin amplification and STR analysis. Both blood and buccal samples spotted onto IsoCode® and IsoCode® ID™ were dried for 15 minutes at 80°C. Three 3mm punches were removed from the sample area and washed in 500ul of water. DNA was eluted into 100ul of water by heating at 95°C for 30 minutes followed by a short burst of pulse vortexing. PCR amplification was performed on the eluates using β-globin as the target gene and K562 human genomic DNA as the positive control. The amplicon generated from the eluates of IsoCode® ID™ and IsoCode® was consistent with the positive control. STR analysis was accomplished by using Promega's PowerPlex® 16 System. One nanogram of DNA eluted from IsoCode® and IsoCode® ID™ was used in the amplification reaction. GeneScan® ver 3.1.2 and GenoTyper® ver2.5 analysis software were used to analyze and type the STR alleles. Identical DNA profiles were obtained from the same saliva sample collected on IsoCode® ID™ and IsoCode®. The average DNA yield from 1, 2 or 3 three mm punches of a buccal sample eluted from IsoCode® ID™ was 10.6ng, 27.6ng and 50.2ng respectively as determined by Applied Biosystem's QuantiBlot® Human DNA Quantitation kit. DNA was eluted from 1, 2 or 3 three mm punches into 30, 60 or 100 ul of water after heating at 95°C for 30 minutes followed by pulse vortexing for one minute. We have demonstrated that IsoCode® ID™ is compatible with several types of DNA polymerases. AmpliTaq®, TaqGold™, FastStart™ Taq, YieldAce™, and *Pfu*Turbo® DNA polymerases displayed robust amplification of a DNA target eluted from buccal samples on IsoCode® ID™. In addition to isolating DNA from buccal samples collected on IsoCode® ID™ by the traditional water elution method, we have also verified the compatibility of IsoCode® ID™ with simple paper-in STR procedures. PowerPlex® 16 STR profiles were obtained by placing 1.2 mm punches of the sample area directly into the amplification reaction. In summary, the results of our study clearly demonstrate that IsoCode® ID™ is functionally equivalent to IsoCode®, and the DNA eluted from oral samples collected on IsoCode® ID™ is of sufficient quantity and quality to use in STR analysis. The ease of identifying the placement of the sample on the matrix makes IsoCode® ID™ especially suited for use with clear oral samples.