

Simple procedure of DNA isolation from human serum

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There have been protocols reported concerning DNA extraction from various biological sources (1–4). A traditional DNA extraction procedure involves DNA separation from the other biological materials by organic extractions, followed by ethanol-precipitation of DNA from the resultant solution (1, 2). We have developed a new simple extraction procedure for DNA purification in human serum in a single tube. The procedure involves simultaneous solubilization of proteins with a high concentration of NaI (chaotropic reagent) and N-lauroyl-sarcosinate, and precipitation of nucleic acids with isopropanol.

The isolated DNA was soluble in buffers, probably due to the residual detergent. The recovery of added DNA at levels in 100 μl human serum exhibited nearly 100% (Figure 1). A serum from a hepatitis B virus carrier was subjected to the procedure and isolated DNA was amplified by PCR (5). Judging from the profile of PCRamplification (Figure 2), the quantity and quality of DNAs isolated by the procedure are better than those prepared by a conventional one (2).

The standard protocol of the procedure is as follows.

1) Dispense 100 μl serum to a 2 ml-microfuge tube with cap.

2) Add 300 μl of a solution containing 6 M NaI/13 mM EDTA/0.5% sodium N-lauroylsarcosine/10 μg glycogen as a carrier/26 mM Tris-HCl, pH 8 to the tube, mix and incubate at 60°C for 15 min in a heating block.

3) Take the tube from the heating block and add an equal volume of isopropanol to the mixture. After vigorously shaking tightly capped tube, stand for 15 min.

4) Centrifuge the tube at 10,000 g × 5 min to precipitate DNA.

5) Add 1 ml of 40% isopropanol and vortex. After centrifugation 10,000 g × 5 min to recover the DNA, the pellet is vacuum-dried. All operations are conducted at RT.

This procedure might be useful for preparation of minute amounts of DNA in biological fluids without loss.

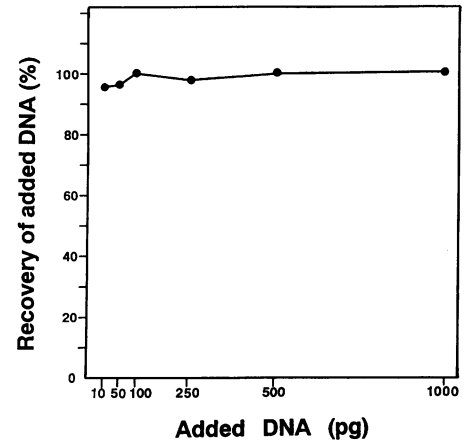

Figure 1. The recovery of added DNA from human serum. Ten to 1,000 pg of HindIII cut lambda-DNA, labeled with [α32p]dCTP by filling reaction, was added to 100 μl of a human serum and the DNAs were extracted with the isolation procedure described here. Radioactivities of the resulted dry pellets were counted and expressed as percentages relative to the original counts.

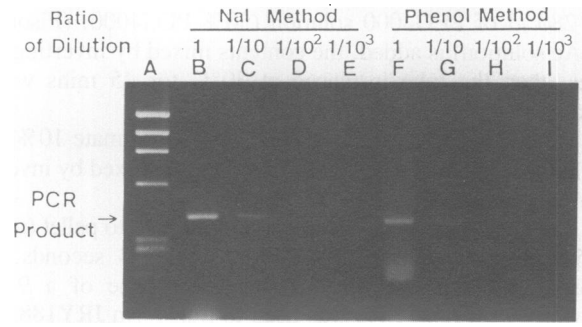

Figure 2. Agarose gel analysis of amplification of segments of HBV surface antigen gene using DNA preparations from HBV carrier serum. A set of serially diluted HBV carrier serum samples with a HBV-free serum were subjected to DNA isolation either by the procedure described here (**NaI Method**) or by the conventional one (**Phenol Method**) (2) as a comparison. Obtained DNAs were PCRamplified as described (5) using 50 pmols of HBV primers and 2.5 unit Taq DNA polymerase (Wako Pure Chemical Industries, Ltd., Japan) with 30 cycles of a rotation of temperatures; 95°C for 1 min, 50°C for 1 min and 72°C for 2 min. Five μl of each sample was electrophoresed on a 1.5% agarose gel. Lane **A:** phix174DNA/HaeIII; Lane **B-E:** amplified products of isolated DNAs by **NaI Method;** Lane **F-I:** amplified products of isolated DNAs by **Phenol Method** (2). Ratios of serial dilution of the HBV serum versus HBV-free serum indicated in **Ratio of Dilution.**

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