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RNAZOL® BD PROTOCOL FOR THE EXTRACTION OF DNA FROM PLASMA OR SERUM.

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The search for physiologically significant markers has spurred interest in the presence of extracellular DNA in circulating plasma or serum. Although RNAzol® BD is designed for the extraction of RNA from blood, this protocol employs alkaline reagent that allows isolation of minute quantities of DNA from plasma or serum samples.

LYSIS

1. Add 1 ml of either plasma or serum to 2 ml of RNAzol® BD.
2. Make sure the tube is tightly closed and vigorously shake the sample - RNAzol® BD mixture for 30 seconds.
2. The lysate may be processed immediately or stored at -20 C prior to DNA extraction.

PHASE SEPARATION

1. Add 50 µl of 6 M NaOH for each ml of plasma or serum in the lysate and mix.
   If samples were frozen, remove the lysate from the freezer and add 50 µl of 6 M NaOH for each ml of plasma or serum in the lysate prior to thawing. Place the tubes in a warm water bath and thaw the contents with shaking until completely thawed.
2. Store the solution at room temperature for 5 minutes.
3. Add 300 µl of BCP (1-bromo-3-chloropropane, MRC Catalog # BP 151) for each ml of plasma or serum in the lysate. Shake vigorously for 30 seconds and store at room temperature for 15 minutes.
4. Centrifuge at 12,000 g for 15 minutes at room temperature.

DNA PRECIPITATION

1. Transfer the clear water supernatant to a clean tube, add 1 - 2 µl of Polyacryl Carrier (MRC Catalog # PC 152) and mix.
2. Add an equal volume of isopropanol and thoroughly mix the solution. Store the sample for 30 minutes at 4 C.
3. Centrifuge at 12,000 g for 15 minutes at room temperature to obtain a pellet with DNA.

DNA WASH

1. Decant or remove the supernatant from the tube without dislodging the DNA pellet. Remove residual solution with a clean pipet tip.
2. Add 1 ml of 75% ethanol and vortex the solution. Store for 2 - 3 minutes at room temperature and centrifuge at 12,000 g for 1 - 2 minutes at room temperature. Decant or remove the wash solution with a pipet tip.
3. Perform a second wash with a fresh aliquot of 75% ethanol and remove all residual wash solution.
4. Centrifuge the pellet for 20 seconds at 12,000 g and carefully remove any residual wash solution.

DNA SOLUBILIZATION

1. Add 20 µl of water and solubilize the DNA pellet for 5 - 10 minutes with periodic vortexing.
2. The expected DNA recovery is below the detection limit of the Nanodrop spectrophotometer. Typically, 2 - 4 ul of the undiluted DNA solution used for PCR yields Ct values ranging from 20 - 40 depending on DNA copy number and primer efficiency and specificity.