Product Application

DNA Purification from Whole Blood Stabilized in Various Blood Collection Tubes using the ReliaPrep™ Blood gDNA Miniprep System

Purify gDNA from fresh blood collected in a variety of blood collection tubes using the ReliaPrep™ Blood gDNA Miniprep System.

Kit: ReliaPrep™ Blood gDNA Miniprep System (Cat. A5081)

Analyses: UV absorbance and QuantiFluor® quantitation, CHEF Gel Electrophoresis

Sample Type(s): Whole blood in ACD, EDTA, Heparin, or PAXgene blood collection tubes

Input: 200µl

This protocol was developed by Promega Applications Scientists and is intended for research use only.

The user is responsible for determining its suitability in the user's application.

Further information can be found in Technical Manual #TM330, available at: **www.promega.com/protocols**

Materials Required:

- ReliaPrep™ Blood gDNA Miniprep System (A5081)
- BD Vacutainer™ Blood Collection Tubes with K_2EDTA (BD Cat.# 366643)
- BD Vacutainer™ Blood Collection Tubes with Sodium Heparin (BD Cat.# 367874)
- BD Vacutainer™ Blood Collection Tubes with ACD (BD Cat.# 364606)
- PAXgene Blood DNA Tubes (Qiagen Cat.# 761115)
- Rotisserie mixer for resuspension of whole blood
- Vortex mixer
- 1.5ml microcentrifuge tubes
- Heating block set to 56°C
- Microcentrifuge capable of 14,000 x g

Protocol

1. Collect fresh whole blood in ACD, EDTA, Heparin, or PAXgene blood collection tubes. Thoroughly mix for at least 10 minutes at room temperature. **Note:** Blood in ACD and PAXgene tubes will be diluted by the stabilization reagent which will result in slightly lower concentrations of blood relative to undiluted samples.
2. Dispense 20µl of Proteinase K Solution into a 1.5ml microcentrifuge tube for each sample.
3. Add 200µl of whole blood to the tubes containing the Proteinase K Solution and briefly mix.
4. Add 200µl of Cell Lysis Buffer to each tube. Cap and mix by vortexing for at least 10 seconds.
5. Incubate at 56°C for 10 minutes.

Proceed with the protocol in the technical manual (TM330) to purify the DNA using the ReliaPrep™ minicolumn.

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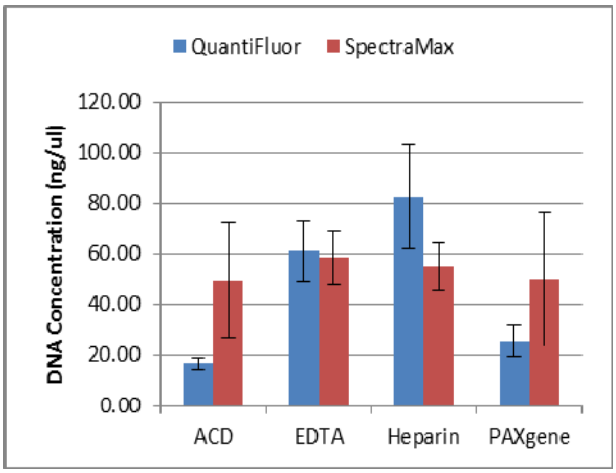
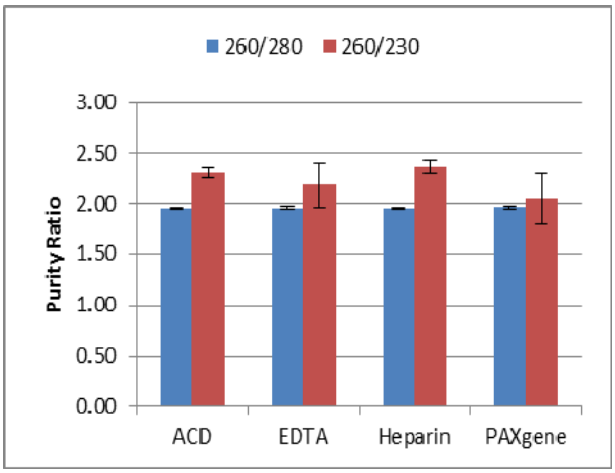
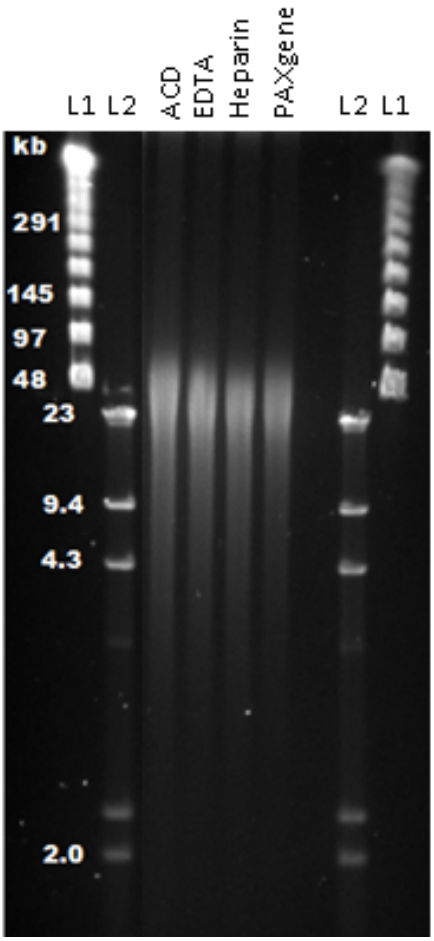
Results:







Top Left: Concentration of eluates (100µl) was determined by the QuantiFluor® ONE dsDNA System and UV absorbance spectroscopy using a Spectramax spectrophotometer. Values are not corrected for blood dilution in ACD and PAXgene tubes. N=3, standard deviations are shown. DNA concentration will vary depending on donor white blood cell count.

Top Right: Purity of eluates was determined by UV absorbance spectroscopy using a Spectramax spectrophotometer. N=3, standard deviations are shown.

Bottom Left: Genomic DNA (0.3-0.5ug) was analyzed on a 1% agarose gel using the CHEF Mapper® (BioRad) at 6.0V/cm. Genomic DNA was stained with ethidium bromide and compared to two molecular weight ladders, L1: Lambda Ladder (NEB), L2: Lambda DNA/HindIII Markers (Promega).