

Isolation of genomic DNA from buffy coat using the QuickPick™ gDNA kit

KEY WORDS: buffy coat, genomic DNA, 20 minute protocol, PickPen®

ABSTRACT

The QuickPick™ gDNA kit is intended for use with human whole blood and blood components such as leukocytes and buffy coat, as well as human cultured cells. Below is described the purification of DNA from buffy coat.

INTRODUCTION

Buffy coat is the layer formed when centrifuging a blood sample. After centrifuging, there are three layers, the upper layer is plasma, the middle layer is buffy coat, and the bottom layer is red blood cells. The buffy coat is composed mainly of leukocytes containing the genomic DNA.

PRINCIPLE OF QuickPick gDNA

DNA in the sample is released from cells using Proteinase K and Lysis Buffer. The released DNA is bound specifically to the magnetic particles in the presence of Binding Buffer. PickPen® 1-M is used to capture the magnetic particles with bound DNA, and to carry out subsequent washes to remove contaminants. Finally, DNA is eluted from the particles using Elution Buffer, and DNA is ready for use in downstream applications. The protocol is carried out in 20 minutes, and throughput can be further increased by using PickPen® 8-M.

MATERIALS & METHODS

Buffy coat was prepared by centrifuging 2 ml of fresh whole blood for 10 minutes at 2500 g at room temperature. From 5 µl to 40 µl of the buffy coat layer was used to isolate genomic DNA, following the QuickPick™ gDNA protocol as described in the kit insert.

RESULTS

The isolated DNA was loaded onto a 1% agarose gel. Intact high molecular weight DNA was detected from all samples applied to the gel.

M 1 2 3 4



M = Marker

1 = DNA isolated from 5 µl of buffy coat

2 = -----"----- 5 µl -----"-----

3 = -----"----- 10 µl -----"-----

4 = -----"----- 40 µl -----"-----