

RNA isolation from blood using the ReliaPrep™ RNA Tissue Miniprep System

To isolate high quality, amplifiable RNA from blood buffy coat fractions using the ReliaPrep™ RNA Tissue Miniprep System.

Kit: ReliaPrep™ RNA Tissue Miniprep System (Cat.#Z6111)

Analyses: RT-qPCR, bioanalyzer

Sample Type(s): Fresh whole blood in EDTA collection tubes

Input: up to 1ml whole blood

Materials Required:

- 100% isopropanol, RNase-free
- 95% ethanol, RNase-free
- Microcentrifuge

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

Users are responsible for determining suitability of the protocol for their application.

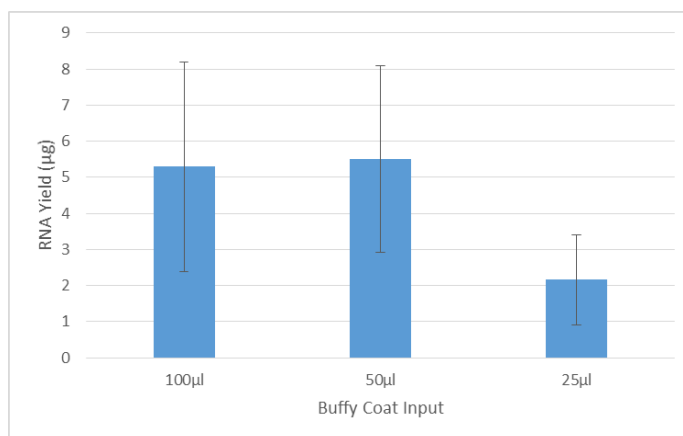
Further information can be found in Technical Manual #TM394, available at: www.promega.com/protocols or please contact techserv@promega.com

Protocol:

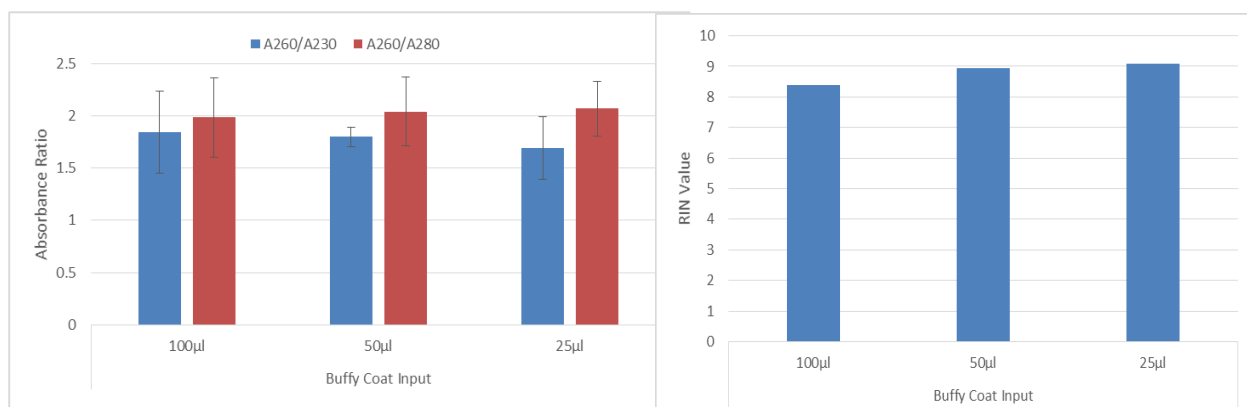
1. Prepare solutions as indicated in the ReliaPrep™ RNA Tissue Miniprep System Technical Manual (TM394).
2. Prepare buffy coat fraction from fresh whole blood by centrifuging the collection tubes for 10 minutes at $2,500 \times g$, and collect the cells at the interface between the lower red blood cell layer and upper plasma layer.
3. Add 500µl of LBA + Thioglycerol solution to the collected buffy coat, vortex briefly and pipet several times to shear genomic DNA.
4. Add 175µl of isopropanol and mix by vortexing for 5 seconds.
5. Proceed with the protocol in the technical manual (TM394) to purify the RNA using the ReliaPrep™ minicolumn.

Product Application

Results: RNA was isolated from 100, 50, and 25 μ l buffy coat samples using the protocol above. Buffy coat was prepared by collecting fresh whole blood in EDTA tubes followed by centrifugation and buffy coat isolation.



RNA yield as determined by RT-qPCR (Above). RNA yield was determined by RT-qPCR with RNA specific primers using a human RNA standard curve. Shown is the mean \pm STD of n=2 for each sample input.



Absorbance ratios from RNA eluates isolated from buffy coat samples (Above Left). Absorbance ratios measured by spectrophotometry on a NanoDrop 1000. Shown is the mean \pm STD of n=2 for each condition.

Quality analysis of RNA eluates isolated from buffy coat samples (Above Right). RNA quality was measured on an Agilent Bioanalyzer and reported as RIN values (10 being the highest possible value).