Optimized Capture and Purification of Nucleic Acid Using the EasySep™ Total Nucleic Acid Extraction Kit, A Robust Magnetic Particle-Based Extraction System

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INTRODUCTION

Effective nucleic acid extraction is fundamental to molecular biology, with successful capture and purification required to study the molecular basis of biological activity. Extraction methods must recover high yields of genetic material while maintaining their purity to facilitate downstream assays including PCR, qPCR, and next-generation sequencing. Many extraction protocols rely on the lysing and nuclease-inactivating properties of a chaotropic salt in the presence of a silica substrate that can bind nucleic acids. Typically, the silica is arranged as a mesh in a spin column, in which the lysate is spun through the column using a centrifuge. Here, we developed EasySep™ Total Nucleic Acid Extraction Kit, in which silica-coated magnetic particles serve as the substrate and a magnet is used to separate the bound nucleic acids from solution. Silica-coated magnetic particles provide an opportunity to improve upon commonly used DNA and RNA extraction protocols with the main advantages being increased yields, the lack of centrifugation steps, as well as flexible and scalable

METHODS

Nucleic Acid Extraction: Figure 1 provides an overview of the standard extraction protocol and an optimized whole blood protocol. Briefly, lysis buffer and Proteinase K are added to cells in suspension and incubated at 56°C for 10 minutes. Magnetic, silica-coated RapidSphere™ particles are then added and incubated at room temperature (RT) for 5 minutes for the liberated DNA and RNA to bind to the particles. The sample is then placed in an ErythroClear™ magnet and the supernatant is discarded. After three washes with ethanol, samples are removed from the magnet and the pellet is resuspended in an elution buffer and incubated at RT for 5 minutes. Lastly, the particles are magnetically removed from the eluted nucleic acid sample, which is then transferred to a fresh tube for downstream applications or storage. Figure 1B provides an overview of the optimized whole blood protocol, which consists of additional separation steps to ensure optimal purities from this complex sample type. The standard protocol has been optimized for medium- and high-throughput (e.g. 96-well) magnetic platforms.

Nucleic Acid Quantification: The primary outputs for assessing nucleic acid extraction efficiency are yield and purity. Following the extraction, sample concentrations were determined through absorbance measurements using a NanoDrop™ 2000 spectrophotometer. Nucleic acid absorbance was used to measure concentration, and ratios for A260/230 or A260/280 were calculated to assess purity. The A260/280 ratio, indicative of protein contamination within the extract, has an optimal range of 1.7 - 1.9. The A260/230 ratio, indicative of salt and phenol contamination, has an optimum of > 1.8. Ratios outside of these ranges are generally considered suboptimal. To determine proportions of DNA and RNA, the Qubit™ Flex Fluorometer was used with Quant-iT™ Assay Kits that detects nucleic acid from 0.2 - 1000 ng. DNA and RNA detection kits were used to quantify each species of nucleic acid in each sample in ng/µL. Finally, qPCR was performed to determine the minimum input amount that could be extracted through serial dilutions using a DNA-based primer probe set complementary to a region on chromosome 4. Amplification was carried out using PrimeTime™ Gene Expression Master Mix (IDT) using manufacturer-recommended cycling conditions and analyzed on the QuantStudio™ 5 Real Time PCR System.

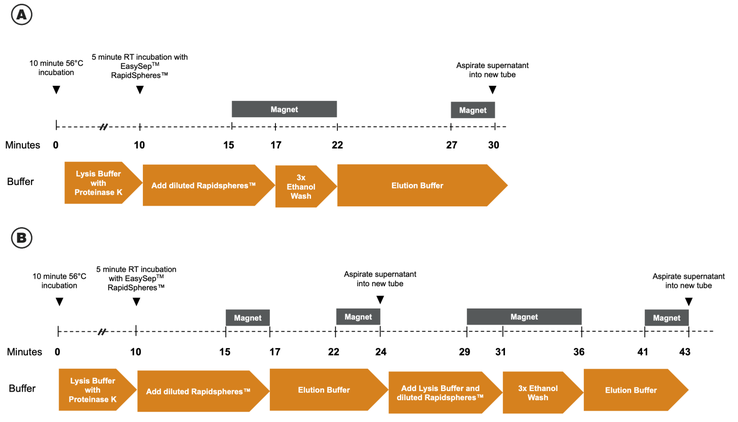

FIGURE 1. EasySep™ Total Nucleic Acid Extraction Protocols

Diagrams of the **(A)** standard and **(B)** whole-blood extraction protocols, with magnetic separation steps indicated by gray boxes.

RESULTS

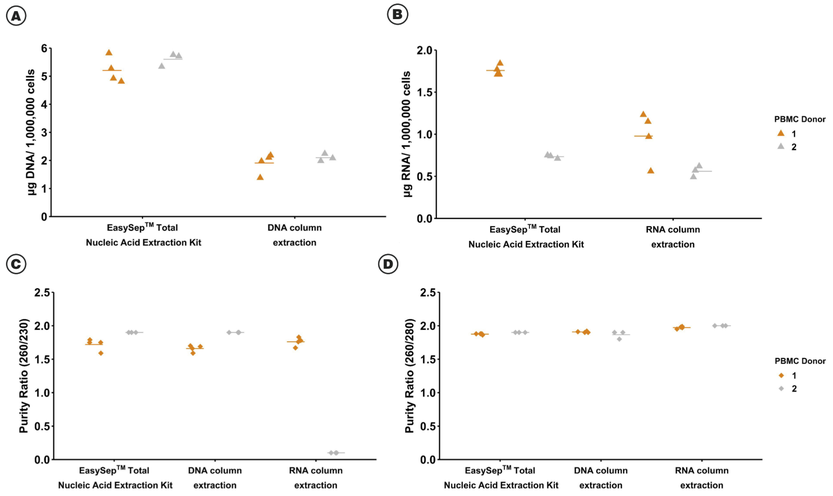

FIGURE 2. EasySep™ Total Nucleic Acid Extraction Kit Shows Improved DNA and RNA Recovery Relative to Other Commonly Used Methods While Maintaining Optimal Purity.

Normalized nucleic acid recovery (i.e. µg/1,000,000 cells) across EasySep™ Total Nucleic Acid Extraction Kit (standard protocol) and spin column-based extraction methods. DNA and RNA concentrations measured separately using the Qubit™ Fluorometer. **(A)** Normalized DNA recovery, **(B)** Normalized RNA recovery. Purity ratios obtained using spectrophotometric absorbance measurements show that the recovered nucleic acid from the EasySep™ Total Nucleic Acid Extraction Kit is of optimal purity, benchmarked against spin column-based protocol. **(C)** 260/230 ratio, **(D)** 260/280 ratio. Sample source: peripheral blood mononuclear cells (PBMCs), 2 individual donors, 3-4 extraction replicates per donor. Horizontal line represents the donor mean within the given condition.

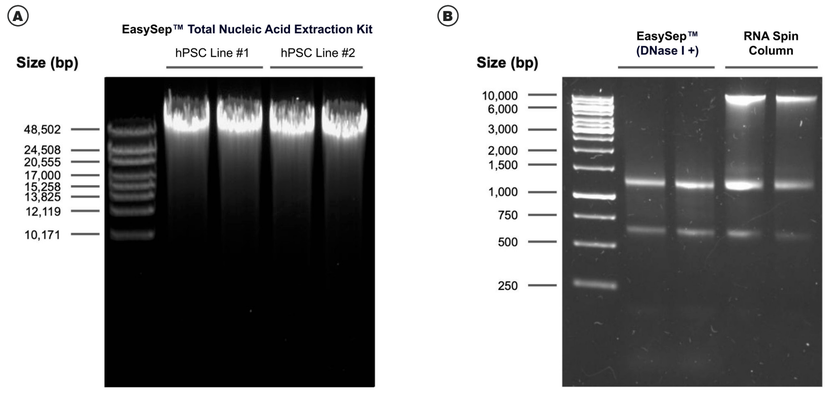

FIGURE 3. Extracted DNA and RNA Show Minimal Degredation When Assessed by Gel Electrophoresis

(A) Gel electrophoresis analysis of 2 human pluripotent stem cell (hPSC) lines reveal that extracted DNA is of high molecular weight (HMW), with a majority of fragments > 48 kilobases (kb). 2 extraction replicates per condition. 0.4% agarose gel in Tris-acetate-EDTA (TAE) buffer. **(B)** RNA gel analysis shows distinct 28S and 16S ribosomal RNA (rRNA) bands at the expected 2:1 intensity ratio, indicative of optimal RNA integrity, benchmarked against RNA spin column extractions. hPSC culture, 2 extraction replicates per condition. 1% agarose gel in TAE buffer. 200 ng of RND loaded per condition.

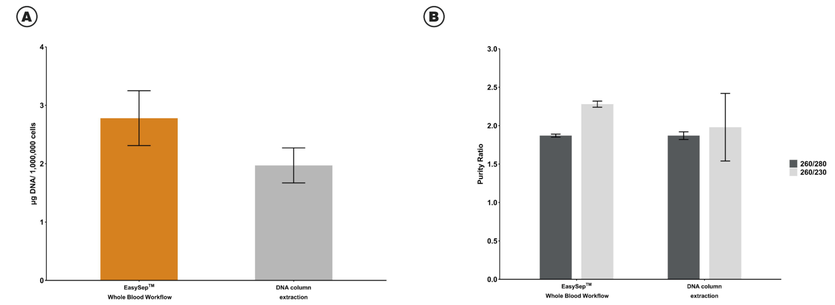

FIGURE 4. Specialized Whole Blood EasySep™ Total Nucleic Acid Extraction Protoocls Shows Superior Recovery Compared to Spin Columns.

(A) Normalized nucleic acid recovery (µg/1,000,000 cells) achieved with EasySep™ Total Nucleic Acid Extraction Kit (whole blood protocol) versus a standard silica mesh spin column protocols. Sample source: whole blood, n = 3. Error bars represent ± 1 standard deviation. **(B)** Purity ratios obtained using spectrophotometric absorbance measurements indicate that the recovered DNA using the specialized whole blood EasySep™ Total Nucleic Acid Extraction Kit protocol is of optimal purity, benchmarked against typical silica mesh spin columns.

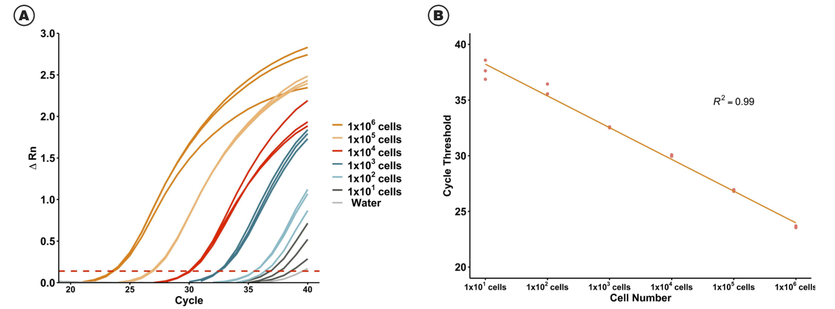

FIGURE 5. EasySep™ Total Nucleic Acid Extraction Kit Can Extract Nucleic Acids From as Few as 10 Cells.

Results from a DNA-based qPCR assay using primers targeted to a non-genic region of chromosome 4 demonstrate that DNA is detectable in extractions with a starting cell input of 10 cells and up to 1,000,000 cells. **(A)** qPCR curves coloured by cell input. Dotted red line represents Ct threshold (0.139). Each individual curve represents 1 technical replicate, n = 3. **(B)** Inverse linear relationship between log (cell number input) and cycle threshold. Each data point represents 1 technical replicate, n = 3. Sample source: PMBCs.

TABLE 1. Summary of Compatible Input Samples by Protocol Type.

Sample Type	Protocol(s)	Optimal Purity	Comments
hPSCs	Standard, 96-well	✓	Tested across 7 PSC lines.
PBMCs	Standard, 96-well	✓	
Whole Blood	Whole Blood	✓	Whole blood is processed directly without the need for red blood cell lysis
3D Hepatic Organoids	Standard	✓	Approximately 1000 organoid fragments per dome. Extracted from 1 - 3 Matrigel® domes per extraction.
EasySep™-separated PBMCs	Standard	✓	
Human Bronchial Epithelial Cells	Standard	✓	Primary cells isolated from whole cadaveric lung, cultured as airway epithelia using PneumaCult™-EX Plus medium.

Summary

- EasySep™ Total Nucleic Acid Extraction kit is a magnetic-based extraction kit compatible with a wide variety of input sample types and sample input amounts.
- This kit consistently generates improved yields with high purities for both DNA and RNA-based applications without the need for centrifugation.
- This kit can extract from whole blood directly without the need for any pre-treatment steps, enhancing ease-of-use for whole blood-based applications.
- The protocol features both a medium-throughput (16-sample) and high-throughput (96-sample) protocol to fit the scale of many different experiment types.