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LONG TERM RNA RECOVERY FROM A REVERSIBLE POROUS MATRIX STORAGE PLATE

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Reversible Porous Matrix (RPM) plates are optimized for storage of very low quantities of pure or slightly impure nucleic acids. The Reversible Porous Matrix is formatted into a 96-well polypropylene, round-bottom plate that facilitates the drying of the matrix into a 2-mm diameter white ceramic disk at the bottom of the well. Reversible Porous Matrix can accommodate RNA samples from 1-ng to 100-ng and can be added in volumes up to 100-µL. RNA samples are eluted from the dried Reversible Porous Matrix by the addition of 20- to 50-µL molecular biology grade nuclease-free water and pipette mixing, after which the Reversible Porous Matrix is removed from the recovered RNA by centrifugation which pellets the RPM and the supernatant containing the RNA is removed and is ready for downstream nucleic acid applications.

Two total RNA samples were used for assessing the ability to recover RNA in sufficient quantity and quality for both RT-PCR and real-time quantitative PCR analysis, from RPM storage particles. Sample one was a purchased fetal liver total RNA with no added RNA stabilizing agents, while sample two was pooled total RNA from extracted 50-uL bloodstains obtained from three male newborn (1-day old) individuals, solubilized in an RNA stabilizing solution. Varying amounts of each RNA sample 10-, 25-, 50-, and 100-ng, concentrated in 25-µL nuclease-free water, was added to the RPM slurry (in duplicate), and the plates were allowed to dry at room temperature in brittan bags containing desiccant pouches. The dried RPM storage plates were sealed with an adhesive cover and stored at either room temperature or at 56C, to simulate "accelerated" extended interval storage conditions. After 1-, 3-, 7-, 14-, 21-, and 28-days of incubation, the RNA was eluted from the RPM plate, quantified, reverse-transcribed and amplified by both PCR and qPCR duplex reactions.

For the gel based RT-PCR duplex assay, a housekeeping gene (GNAS) and a sample specific gene of interest (HBG2n3) were the amplification targets; while the real-time PCR duplex assay identified, a housekeeping gene, the ribosomal protein S15 and a sample specific gene of interest, a gamma hemoglobin isoform, HBG1n1. Our results illustrated that high quality and sufficient quantity of RNA was consistently recovered from both RNA samples at all time points from both storage conditions. The RT-PCR and real-time PCR amplification reactions yielded two distinct bands after gel electrophoresis and cycle threshold values, respectively, in all tested samples, at all time points, with both storage conditions. The only samples that failed to amplify were the 10-ng fetal liver RNA input samples in the RT-PCR based assay with the 56C storage conditions at days 21 and 28 of storage.

The results obtained in this study illustrated that pure or slightly impure RNA could be successfully recovered from extended periods of storage, with the use of a Reversible

Porous Matrix storage vesicle, for use in both RT-PCR gel based and quantitative real-time PCR based amplification duplex reactions.