# Quantitative RT-PCR analysis of human monocytes using total RNA purified using the BioRobot® M48 workstation

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This study describes the suitability of total RNA purified using the BioRobot® M48 workstation for sensitive RT-PCR applications. Numbers of gene transcripts correlated well between samples containing different numbers of cell equivalents, indicating highly pure RNA and consistent purification efficiency. Ratios of target transcripts relative to a housekeeping gene correlated well, independent of cell numbers.

## Introduction

Examining the differential transcription of genes in populations of cells helps decipher the complex mechanisms involved in cellular interactions, migration, and differentiation. To accurately measure gene expression in cell populations, it is important to use a reliable and standardized purification method that gives representative yields of RNA from a range of cell counts. Purified RNA must also be of high quality, and free from contaminating genomic DNA.

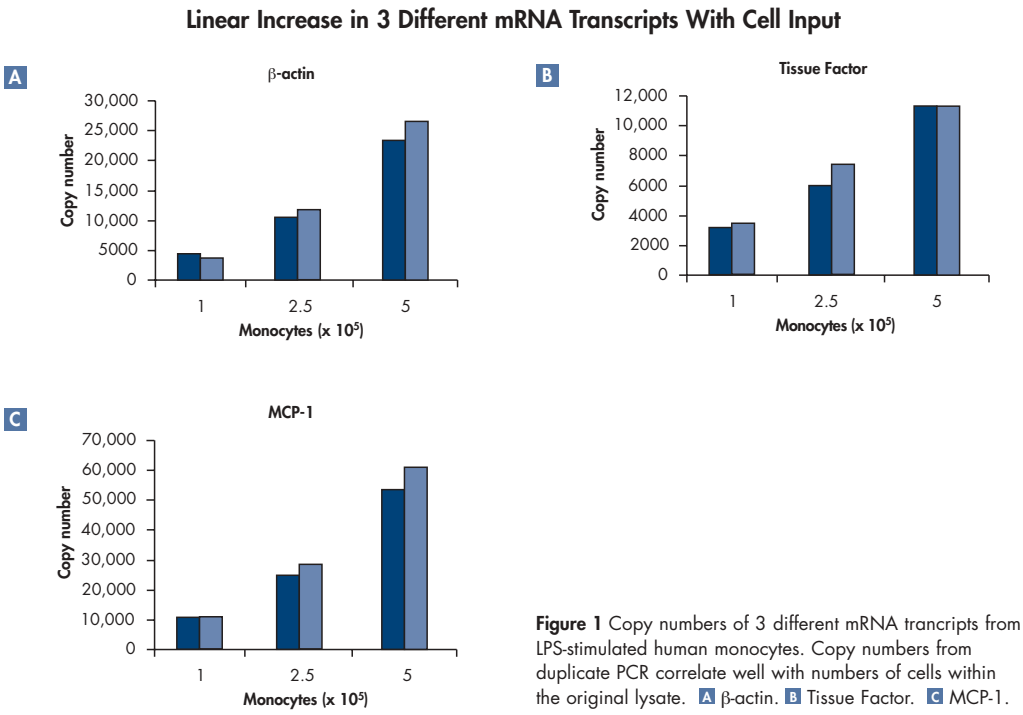


**Figure 1** Copy numbers of 3 different mRNA trancripts from LPS-stimulated human monocytes. Copy numbers from duplicate PCR correlate well with numbers of cells within the original lysate. A β-actin. B Tissue Factor. C MCP-1.

## Materials and methods

Human monocytes were extracted from whole blood by density gradient centrifugation, quantified by flow cytometry, and activated by incubation with LPS (1). Cells were lysed according to the MagAttract® RNA Cell Mini M48 Kit protocol and the lysate diluted to yield aliquots equivalent to 1 x $10^5$, 2.5 x $10^5$, and 5 x $10^5$ human monocytes. Purification of total RNA from lysates (400 µl) was fully automated using the BioRobot M48 workstation with the MagAttract RNA Cell Mini M48 Kit. DNase digestion was performed on the workstation and total RNA was eluted in 200 µl.

cDNA was transcribed in 20 µl reactions, combining 8 µl RNA and 12 µl RT mastermix. DNA was amplified from 2 µl cDNA in a 20 µl PCR using primers specific for MCP-1, Tissue Factor, and β-actin (2). PCR was performed in duplicate and control PCR was performed from sham RT reactions to ensure the absence of genomic DNA. Transcript numbers were read from transcript-specific standard curves (2).

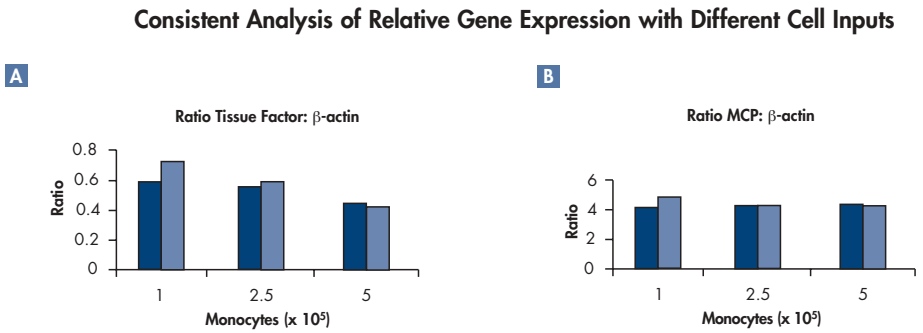


**Figure 2** Ratios of mRNA copy numbers relative to β-actin mRNA in increasing numbers of LPS-stimulated human monocytes. Ratios from duplicate PCR and also from increasing numbers of cell equivalents, correlate well. A Tissue Factor: β-actin. B MCP-1: β-actin.

## Results

RT-PCR analyses showed accurate quantification of mRNA transcript numbers that accurately correlate to numbers of cell equivalents in the original sample (Figure 1). Transcript copy numbers were calculated from a calibration curve generated using defined standards of known copy-number (2). Ratios of MCP-1 and Tissue Factor transcripts relative to the housekeeping gene β-actin correlate well, independent of cell input (Figure 2).

## Conclusion

The BioRobot M48 workstation in combination with the MagAttract RNA Cell Mini M48 Kit provides standardized, fully automated purification of total RNA from cells.

- RNA is purified from a wide range of cell inputs with consistent efficiency and purified RNA performs reliably in sensitive RT-PCR analysis.
- Highly pure RNA enables reliable RT-PCR, which allows sensitive and accurate gene expression analyses.

**References**


1. Lund, P.K., Joo, G.B., Westvik, A.B., Ovstebo, R., Kierulf, P. (2000) Isolation of monocytes from whole blood by density gradient centrifugation and counter-current elutriation followed by cryopreservation: six years' experience [In Process]. Scand J Clin Lab Invest. **60**: 357.
2. Ovstebo, R., Haug, K.B., Lande, K., Kierulf, P. (2003) PCR-based calibration curves for

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