Direct lysis of 3D cell cultures for RT-qPCR gene expression quantification

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July 5, 2022

Abstract

In vitro cell culture experiments are widely used to study cellular behavior in most biological research fields. Except for suspension cells, most human cell types are cultured as adherent monolayers on a plastic surface. While technically convenient, monolayer cultures can suffer from limitations in terms of physiological relevance, as their resemblance to complex in vivo tissue structures is limited. To address these limitations, three-dimensional (3D) cell culture systems have gained increased interest as they mimic key structural and functional properties of their in vivo tissue counterparts. Nevertheless, protocols established on monolayer cell cultures may require adjustments if they are to be applied to 3D cell cultures. As gene expression quantification is an essential part of many in vitro experiments, we evaluated and optimized a direct cell lysis, reverse transcription and qPCR protocol applicable for 3D cell cultures. The newly developed protocol wherein gene expression is determined directly from crude cell lysates showed improved cell lysis compared to the standard protocol, accurate gene expression quantification, hereby avoiding time-consuming cell harvesting and RNA extraction.

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