Cell Culture Supernatant Collection

Louise C. Laurent (llaurent@ucsd.edu)
University of California San Diego, Sanford Consortium for Regenerative Medicine, 2880 Torrey Pines Scenic Drive, La Jolla, CA

Roger P. Alexander (rogerpalexander@gmail.com)
Sample and Assay Standards Working Group of the Extracellular RNA Communication Consortium

Method Article

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Abstract

This protocol describes how to collect supernatant from a cell culture preparation in order to detect, identify and quantify extracellular RNA from the sample.

Introduction

Extracellular RNAs \(exRNAs) have been identified in every biofluid that has been tested. They have been found in extracellular vesicles, ribonucleoprotein complexes and lipoprotein complexes. exRNAs are interesting because they may serve as signalling molecules between cells, they have the potential to serve as biomarkers for prediction and diagnosis of disease, and exRNAs or the extracellular particles that carry them might be used for therapeutic purposes. The Sample and Assay Standards Working Group of the Extracellular RNA Communication Consortium \(ERCC) is a group of laboratories funded by the U.S. National Institutes of Health to develop robust and standardized methods for collecting and processing of biofluids, separating different types of exRNA-containing particles and isolating and analyzing exRNAs. In our first joint endeavour, we held a series of conference calls and in-person meetings to survey the methods used among our members, placed them in the context of the current literature and used our findings to identify areas in which the identification of robust methodologies would promote rapid advancements in the exRNA field. A full list of the protocols developed during this effort is available at the exRNA Portal, the ERCC's website \("http://exrna.org/resources/protocols/":http://exrna.org/resources/protocols/). This protocol for collecting cell culture supernatant is one of the biofluid collection and processing methods compared in "the associated publication":http://www.journalofextracellularvesicles.net/index.php/jev/article/view/26533.

Procedure

This protocol has been performed using human embryonic stem cells \(hESCs), neonatal rat ventricular myocytes \(NRVMs), and KMBC cholangiocarcinoma cells. 1. On Day 1, plate cells at a concentration of 1 million cells / mL. 2. 24 hours after plating, switch to serum-free or EV-cleared media. 3. Collect supernatant after 24 hr \(hESCs) or 48 hr \(NRVMs or KMBCs). 4. Centrifuge at 500 x g for 10 min. 5. Transfer supernatant to a fresh tube and centrifuge at 2,000 x g for 10 min. 6. Store cell-free supernatant at -80°C.