emulate

Protocol for Emulate Organ-Chips:

Alkaline Phosphatase (ALP) Assay

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EP199 v1.0

TITLE	DOCUMENT	VERSION
Alkaline Phosphatase (ALP) Assay	EP199	1.0
	DATE	PAGE
	17-JUL-2019	2 OF 2

Goals:	Key Steps:	Other Required Materials:
Quantify Alkaline Phosphatase (ALP) activity from Emulate Organ-Chip lysate samples	• Prepare all reagents, samples, and standards • Run the assay • Read assay plate(s) immediately	• AttoPhos® AP Fluorescent Substrate System (Promega, S1000 or S1001) • Plate reader

Introduction

Alkaline phosphatases (ALPs) are a family of cell surface glycoproteins with ALP isoenzymes expressed in kidney as well as in a variety of other organs such as liver, intestine, and bone. In the clinic, elevated levels of serum ALP are associated with disease or injury in serval organs. It can be applied as a marker of kidney injury and disease. This protocol uses the Proximal Tubule Kidney-Chip as a reference point, and these assay conditions could change with a different Organ-Chip.

Method

Sample type	Organ-Chip lysate samples See Emulate Protocol EP135 Cell Lysis for Protein Extraction.
Recommended assay flow rate (Proximal Tubule Kidney-Chip)	60 µL / hr
Recommended effluent dilution (Proximal Tubule Kidney-Chip)	No dilution, samples are loaded neat. Note: ALP levels will change depending on cell injury status or based on donor-to-donor variability. Therefore, sample dilutions may need to be modified to accommodate different experimental conditions or cells from different donors.
Run assay as described on supplier site	https://www.promega.com/products/protein-expression/protein-labeling-and-detection/attophos-ap-fluorescent-substrate-system/?catNum=S1000 Note: Store all components of the AttoPhos® AP Fluorescent Substrate Kit at 4°C protected from light.