

Summary Report

Project Number

GIFL002STU

Report Title

Evaluation of Human Cells Isolated from Amniotic Membrane
by Flow Cytometry

Test Site



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Sponsor



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INTRODUCTION

The objective of this project was to evaluate human cells isolated from amniotic membrane by flow cytometry.

PROJECT SCHEDULE

Sample Analysis Start	February 08, 2018
Sample Analysis Completion	February 08, 2018

MATERIALS AND METHODS

Equipment and Materials

This is a list of the major equipment and materials that were used on this project. Additional equipment was documented in the study paperwork and will be maintained with the study data.

Table 1: Equipment and Materials

Item	Supplier
Guava PCA with CytoSoft software	EMD Millipore
Water Bath	VWR
BD 10-color FACSCanto™ with FACSDiva software	BD

Reagents

This is a list of the specific reagents that were used on this project.

Item	Supplier	Clone
1X DPBS CMF	Life Technologies	NA
Stain Buffer (BSA)	BD	NA
Isotypes	BioLegend or BD	NA
CD45 V510	BioLegend	HI30
CD29 AF488	BioLegend	TS2/16
CD44 AF700	BioLegend	G44-26
CD34 PE	BD	563
CD166 PerCPCy5.5	BD	3A6
CD105 APC	BD	266
CD90 PECy7	BD	5E10
CD73 V450	BD	AD2
Live/Dead Stain NIR	Life Technologies	NA

Sample Receipt and Disposal

Frozen specimens, 3 vials, were shipped on dry ice and stored in liquid nitrogen until testing. All processed specimens were disposed after data acquisition was complete.

Cell count and Viability

Specimen ID	FCS Lab ID	Viability (%)	Total Cells (Cells/ml)	Viable Cells (Cells/ml)
BTR180407 vial 1	001A	72.3	5.20 x 10 ⁶	3.7 x 10 ⁶
BTR180407 vial 2	001B	85.8	4.22x 10 ⁶	3.6 x 10 ⁶
BTR180407 vial 3	001C	69.5	5.27 x 10 ⁶	3.6 x 10 ⁶

MEAN/AVERAGE	75%	4.89 x 10⁶	3.6 x 10⁶
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Cell surface markers expression

The following cell surface markers were selected because these are characteristic markers of Mesenchymal Stem Cells. The presence of the marker is indicated by (+) and the absence of the marker is indicated by (-).

Cell surface Markers	Function	Expression
CD90 (Thy-1)	One of the most heavily glycosylated membrane proteins. Thy-1 can be used as a marker for a variety of stem cells	+
CD105 (Endoglin)	Endoglin (CD105) is a component of the transforming growth factor-beta (TGF- β) receptor (TGF- β R) complex.	+
CD166	Transmembrane glycoprotein believed to have a role in cell adhesion, usually used as a marker for a variety of stem cells.	+
CD73	CD73 has enzymatic and non enzymatic properties. As a nucleotidase, CD73 catalyzes the hydrolysis of AMP into adenosine and phosphate, very important property for immunoescape activity. Non enzymatically, CD73 is also a signal and adhesive molecule that can regulate cell interaction with extracellular matrix (ECM) components, such as laminin and fibronectin.	+
CD29 (Integrin— β 1)	Involved in cell adhesion.	+
CD44 (HCAM)	Involved in cell-cell interactions, cell adhesion and migration.	+

The following hematopoietic markers were selected to test for blood contamination. The presence of the marker is indicated by (+) and the absence of the marker is indicated by (-).

Cell surface Markers	Function	Expression
CD34	Characteristic marker of hematopoietic stem cells.	-
CD45	Lymphocyte common antigen characteristic of white blood cells	-