

**LABORATORY NOTEBOOK**

030





# LABORATORY NOTEBOOK

Company Name: CMRL



Assigned To: Denise Hsu

Department: BME



Notebook No: 030

## INSTRUCTIONS

1. This notebook and all the information recorded therein are the sole property of this Company. The contents of this notebook are strictly confidential and may be disclosed to others only with the written permission of the Company. The employee must return this notebook upon request or termination of employment. Keep this notebook in a protected place to prevent loss. In the event of loss, notify your supervisor immediately and draft a written statement describing the contents of the notebook and the manner in which it was lost.
2. This notebook is intended to be a permanent record of your lab or field work. In order to fully protect your work and achieve the desired recognition, either academic or economic, you will need to pay careful attention to the manner in which you record entries in this notebook.
3. Write in concise and clear language and write everything down. Draw and diagram directly on these pages. To include a printout: label the printout, attach it securely to the notebook page, and then write a brief description of the printout in the notebook directly below the place where it is attached. All note should be made in the book, not on loose pages stuck inside the notebook. Be clear and safeguard your work.
4. Use only ink when marking in this notebook. Pencil markings should be avoided. To delete an error: draw a single line through the error and place your initials and the date close by.
5. The title, date and project name should be recorded at the start of each entry. Make sure you write down your full name, lab location and company or institution in the front of the notebook.
6. Begin your entries by explaining in chronological order exactly what procedure were used and in what order you completed the various steps. Date your entries. Specify the equipment and consumable materials that were used, preferably by manufacturer and part number. Describe completely and accurately exactly what results were achieved, and at what stages, including the time. It is always better to include too much detail in your entries, than too little. If you make a scientific discovery, or invent a product, these details may be very important to proving any new discoveries.
7. Witnesses are important in cases where new concepts or approaches are determined. Also, where new discoveries are made, or where the potential exists for a patent. In those cases, at least one witness who is not a co-discoverer should sign and date in the indicated space at the bottom of the relevant work sheets. The witness needs to be able to understand and describe the basic procedures and the results they observed.
8. Patentable subject matter may appear in the course of your work. Any new and surprising product, composition or method may be patentable. Be especially alert for patentability when results appear strange, interesting or of commercial importance. Protect the results by writing in your notebook: a) what the result is, b) why the result is significant, and c) how the result was produced.
9. The time between the actual experiment or procedure and the time that you record your findings should be minimized. Act immediately in order to fully record your findings.
10. Separate your notes for each long term project into separate notebooks. Do not use a single page to record observations or procedures from more than one subject.

Assigned To \_\_\_\_\_ Date \_\_\_\_\_ Notebook No. \_\_\_\_\_

Returned To \_\_\_\_\_ Date \_\_\_\_\_ By \_\_\_\_\_

Transferred To \_\_\_\_\_ Date \_\_\_\_\_ By \_\_\_\_\_

Continued From Notebook Number \_\_\_\_\_ Date \_\_\_\_\_

Continued To Notebook Number \_\_\_\_\_ Date \_\_\_\_\_

Plate1 ARS - Raw

	1	2	3	4	5	6	7	8	9	10	11	12
A	OVRFLW	OVRFLW	OVRFLW	OVRFLW	OVRFLW	OVRFLW	2.314	0.043	0.05	0.051	0.05	0.05
B	0.681	0.689	0.596	2.038	2.017	1.7	0.051	0.052	0.053	0.052	0.051	0.05
C	1.276	1.262	1.068	1.306	1.321	0.949	1.283	1.268	0.928	1.421	1.413	1.107
D	0.57	0.573	0.443	0.792	0.779	0.596	0.585	0.59	0.45	0.657	0.642	0.463
E	0.763	0.768	0.634	1.333	1.327	0.982	0.918	0.925	0.767	1.291	1.277	1.085
F	1.114	1.107	0.798	1.143	1.117	0.897	0.705	0.705	0.512	1.8	1.793	1.388
G	1.964	1.929	1.4	1.781	1.784	1.268	1.885	1.888	1.419	1.631	1.595	1.184
H	1.322	1.29	1.001	0.648	0.66	0.465	0.72	0.733	0.556	2.15	2.148	1.912

Plate1 BCA - Raw

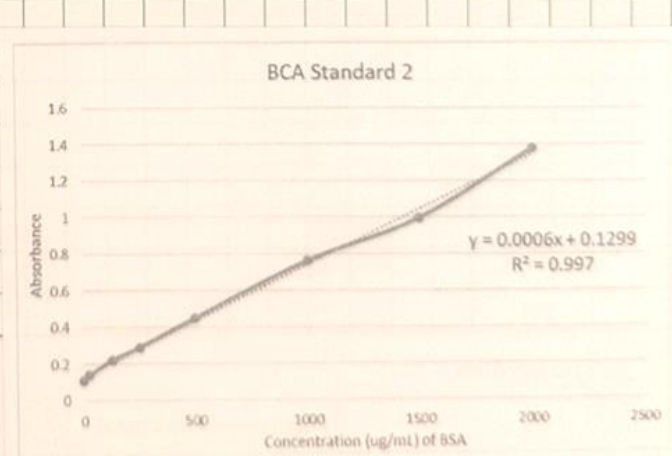
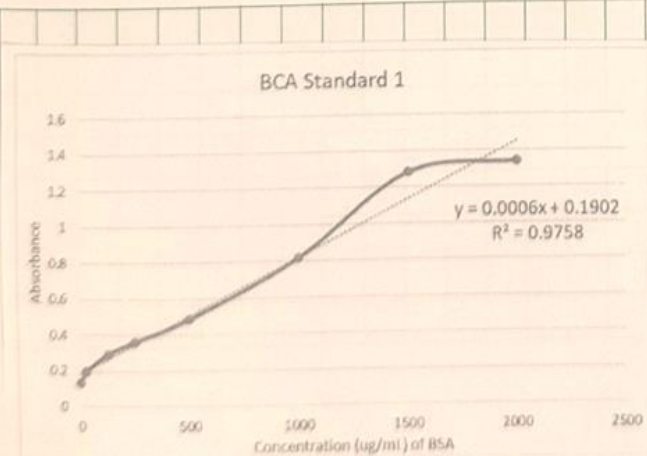
	1	2	3	4	5	6	7	8	9	10	11	12
A	1.341	1.284	0.821	0.489	0.365	0.295	0.2	0.132	0.043	0.043	0.044	0.043
B	0.42	0.603	0.538	0.588	0.601	0.545	0.044	0.043	0.044	0.044	0.044	0.043
C	0.925	1.036	0.955	0.688	0.726	0.725	0.681	0.687	0.696	0.896	0.927	0.918
D	0.543	0.588	0.555	0.376	0.41	0.382	0.544	0.462	0.435	0.387	0.623	0.406
E	0.738	0.848	0.757	0.785	0.919	0.829	0.677	0.677	0.757	0.774	0.828	0.77
F	0.394	0.447	0.414	0.386	0.536	0.456	0.488	0.423	0.337	0.575	0.639	0.558
G	0.605	0.745	0.696	0.752	0.622	0.666	0.551	0.568	0.666	0.647	0.672	0.684
H	0.563	0.585	0.623	0.537	0.661	0.634	0.522	0.486	0.445	0.574	0.608	0.574

Plate2 ARS - Raw

	1	2	3	4	5	6	7	8	9	10	11	12
A	OVRFLW	OVRFLW	3.479	3.028	2.226	1.667	0.815	0.05	0.051	0.051	0.05	0.05
B	1.755	1.782	1.492	0.053	0.053	0.051	0.051	0.052	0.053	0.052	0.051	0.05
C	0.759	0.753	0.616	0.704	0.693	0.541	0.316	0.319	0.232	1.609	1.582	1.467
D	0.742	0.749	0.668	0.704	0.698	0.569	0.549	0.55	0.432	1.005	1.009	0.82
E	0.701	0.707	0.58	0.337	0.342	0.25	0.355	0.356	0.234	1.088	1.065	0.987
F	0.05	0.052	0.053	0.053	0.053	0.052	0.053	0.053	0.053	0.053	0.052	0.05
G	1.341	1.334	1.194	0.584	0.583	0.567	0.438	0.442	0.39	0.36	0.354	0.308
H	0.592	0.596	0.481	0.768	0.766	0.672	0.369	0.37	0.312	0.842	0.837	0.844

Plate2 BCA - Raw

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.378	0.996	0.76	0.449	0.29	0.22	0.139	0.111	0.043	0.043	0.044	0.044
B	0.508	0.548	0.556	0.044	0.044	0.043	0.044	0.043	0.044	0.045	0.045	0.044
C	0.41	0.432	0.434	0.409	0.426	0.43	0.372	0.395	0.385	0.679	0.715	0.723
D	0.55	0.587	0.594	0.496	0.516	0.508	0.501	0.533	0.535	0.555	0.577	0.584
E	0.535	0.556	0.548	0.395	0.451	0.461	0.44	0.414	0.427	0.562	0.61	0.615
F	0.043	0.045	0.045	0.044	0.044	0.044	0.044	0.044	0.044	0.045	0.043	0.044
G	0.447	0.458	0.463	0.366	0.386	0.385	0.379	0.407	0.394	0.364	0.395	0.381
H	0.393	0.416	0.416	0.345	0.371	0.377	0.372	0.399	0.375	0.343	0.377	0.371



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1/9/2022

96-well plate 1

	1	2	3	4	5	6	7	8	9	10	11	12
A	Fresh PC 1	Fresh PC 1	Fresh PC 1	Fresh PC A	Fresh PC A	Fresh PC A	Fresh PC 1	0.25 Org PC 1	0.25 Org PC 1	0.50 Org PC 1	0.50 Org PC 1	0.50 Org PC 1
B	Static Org PC1	Static Org PC1	Static Org PC1	Steady Org PC 1	Steady Org PC 1	Steady Org PC 1	0.25 EX-PC 1	0.25 EX-PC 1	0.25 EX-PC 1	0.50 EX-PC 1	0.50 EX-PC 1	0.50 EX-PC 1
C	Static EX-PC 1	Static EX-PC 1	Static EX-PC 1	Steady EX-PC 1	Steady EX-PC 1	Steady EX-PC 1	0.25 CY-PC 1	0.25 CY-PC 1	0.25 CY-PC 1	0.50 CY-PC 1	0.50 CY-PC 1	0.50 CY-PC 1
D	Static Org PC A	Static Org PC A	Static Org PC A	Steady Org PC A	Steady Org PC A	Steady Org PC A	0.25 Org PC A	0.25 Org PC A	0.25 Org PC A	0.50 Org PC A	0.50 Org PC A	0.50 Org PC A
E	Static EX-PC A	Static EX-PC A	Static EX-PC A	Steady EX-PC A	Steady EX-PC A	Steady EX-PC A	0.25 EX-PC A	0.25 EX-PC A	0.25 EX-PC A	0.50 EX-PC A	0.50 EX-PC A	0.50 EX-PC A
F	Static CY-PC A	Static CY-PC A	Static CY-PC A	Steady CY-PC A	Steady CY-PC A	Steady CY-PC A	0.25 CY-PC A	0.25 CY-PC A	0.25 CY-PC A	0.50 CY-PC A	0.50 CY-PC A	0.50 CY-PC A

96-well plate 2

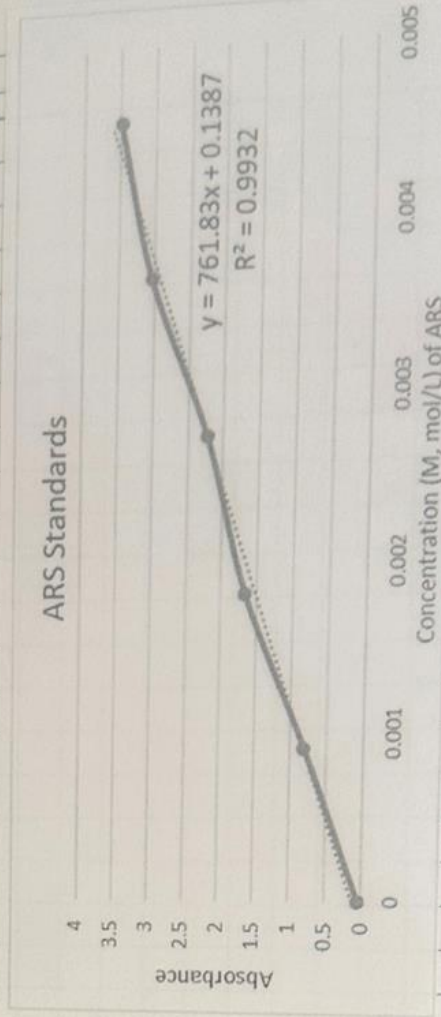
	1	2	3	4	5	6	7	8	9	10	11	12
A	Standard 8	Standard 7	Standard 6	Standard 5	Standard 4	Standard 3	Standard 2	Standard 1				
B	Fresh PC B	Fresh PC B	Fresh PC B	Fresh PC B	Fresh PC B	Fresh PC B	0.25 Org PC B	0.25 Org PC B	0.25 Org PC B	0.50 Org PC B	0.50 Org PC B	0.50 Org PC B
C	Static Org PC B	Static Org PC B	Static Org PC B	Steady Org PC B	Steady Org PC B	Steady Org PC B	0.25 EX-PC B	0.25 EX-PC B	0.25 EX-PC B	0.50 EX-PC B	0.50 EX-PC B	0.50 EX-PC B
D	Static EX-PC B	Static EX-PC B	Static EX-PC B	Steady EX-PC B	Steady EX-PC B	Steady EX-PC B	0.25 CY-PC B	0.25 CY-PC B	0.25 CY-PC B	0.50 CY-PC B	0.50 CY-PC B	0.50 CY-PC B
E	Static Org PC A	Static Org PC A	Static Org PC A	Steady Org PC A	Steady Org PC A	Steady Org PC A	0.25 Org PC A	0.25 Org PC A	0.25 Org PC A	0.50 Org PC A	0.50 Org PC A	0.50 Org PC A
F	Static EX-PC C	Static EX-PC C	Static EX-PC C	Steady EX-PC C	Steady EX-PC C	Steady EX-PC C	0.25 EX-PC C	0.25 EX-PC C	0.25 EX-PC C	0.50 EX-PC C	0.50 EX-PC C	0.50 EX-PC C
G	Static CY-PC C	Static CY-PC C	Static CY-PC C	Steady CY-PC C	Steady CY-PC C	Steady CY-PC C	0.25 CY-PC C	0.25 CY-PC C	0.25 CY-PC C	0.50 CY-PC C	0.50 CY-PC C	0.50 CY-PC C

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Date

GROUP	mmoles/ug	SEM
Fresh PC	0.002637188	0.000359949
Static PC	0.001873615	0.000321546
Steady PC	0.001935328	0.000296043
0.25 PC	0.001296915	0.000222872
0.50 PC	0.002073129	0.000259905
Static EX-PC	0.0018235	0.000289855
Steady EX-PC	0.001795101	0.000194549
0.25 EX-PC	0.001473574	0.000324419
0.50 EX-PC	0.001419089	0.000194826
Static CY-PC	0.001269825	0.000169382
Steady CY-PC	0.001159877	0.000186345
0.25 CY-PC	0.000920445	0.000121013
0.50 CY-PC	0.002296644	0.00029647

ARS Standards

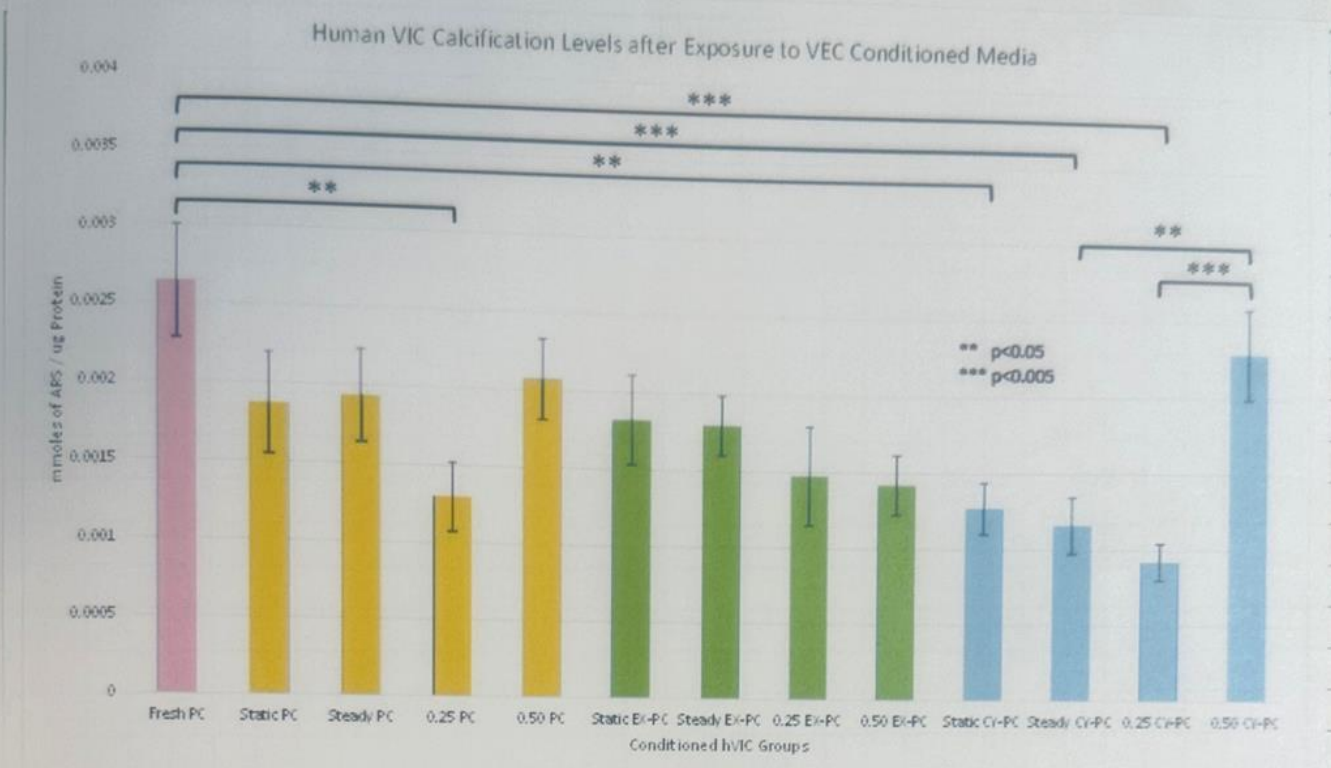


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Date

1/26/2022



1/15/2022: hVIC RNA extraction/quantification

TUBE #

ng/ $\mu$ L



1-1	} FRESH	144.2
1-2		234.4
2-1	} PC	16.8
2-2		45.5
3-1	} static	34.0
3-2		76.2
4-1	} steady	39.7
4-2		116.6
5-1	} 0.25	32.7
5-2		51.8
6-1	} 0.50	74.4
6-2		130.3

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1/26/2022

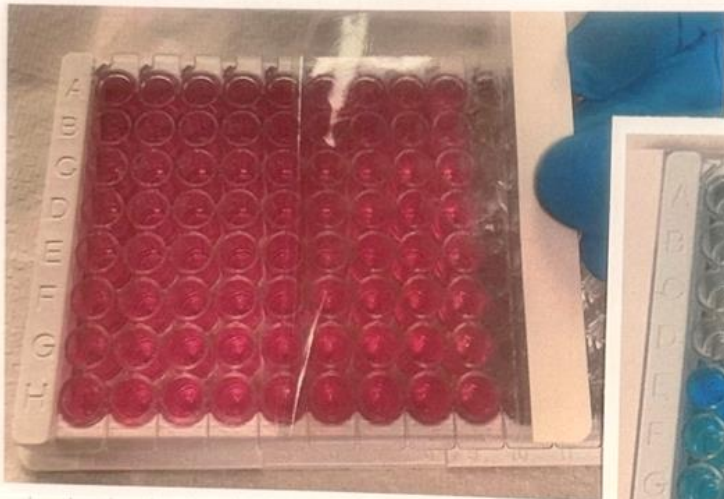


3/12/2022: Cytokine panel (96-well plate, 8 cytokines per strip, total 12 strips)

Fresh PC	Static EX-PC	steady EX-PC	0.25 EX-PC	0.50 EX-PC	Static CY-PC	steady CY-PC	0.25 CY-PC	0.50 CY-PC	X
1	2	3	4	5	6	7	8	9	10-12
A TGFβ									
B VEGF									
C TNFα									
D IL-1β									
E IL-6									
F IL-8									
G MCP-1									
H GM-CSF									

ELISA

protocol on pg. 100 ~ 98



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Date \_\_\_\_\_

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3/12/2022

Date \_\_\_\_\_

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.119	0.117	0.123	0.12	0.112	0.106	0.117	0.119	0.105	0.042	0.043	0.045
B	0.134	0.124	0.142	0.141	0.134	0.133	0.128	0.141	0.134	0.046	0.043	0.048
C	0.094	0.083	0.095	0.092	0.086	0.086	0.087	0.092	0.089	0.044	0.046	0.049
D	0.077	0.067	0.072	0.07	0.07	0.069	0.074	0.076	0.072	0.044	0.044	0.049
E	3.51	3.351	3.352	3.341	3.395	3.571	3.206	3.455	3.619	0.043	0.047	0.043
F	0.566	0.301	0.229	0.289	0.31	1.545	0.231	0.55	1.314	0.044	0.045	0.044
G	0.641	0.543	0.658	0.605	0.461	0.625	0.546	0.543	0.498	0.046	0.049	0.048
H	0.116	0.094	0.103	0.101	0.094	0.094	0.102	0.11	0.121	0.043	0.042	0.043


	1	2	3	4	5	6	7	8	9	10	11	12
Fresh PC	Static EX-PC	Steady EX-PC	0.25 EX-PC	0.50 EX-PC	Static CY-PC	Steady CY-PC	0.25 CY-PC	0.50 CY-PC				
A	TGFb	TGFb	TGFb	TGFb	TGFb	TGFb	TGFb	TGFb	TGFb	TGFb	TGFb	TGFb
B	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF
C	TNfa	TNfa	TNfa	TNfa	TNfa	TNfa	TNfa	TNfa	TNfa	TNfa	TNfa	TNfa
D	IL-1b	IL-1b	IL-1b	IL-1b	IL-1b	IL-1b	IL-1b	IL-1b	IL-1b	IL-1b	IL-1b	IL-1b
E	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6
F	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8
G	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1
H	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF


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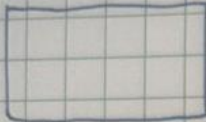
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3/12/2022

6/13/2022: PSIS Scaffold Valve Area Cell Count



81.64 mm

20 mm

→ + three posts' widths at 7 mm per post  
 $81.64 - 21 = 60.64$  mm



$$C = \pi \times 26 \text{ mm}$$

$$C = 81.64 \text{ mm}$$

Valve area



81.64 mm

$$20 \text{ mm} = 1632.8 \text{ mm}^2 = 16.328 \text{ cm}^2$$

$$\frac{2 \text{ million cells}}{2.5 \text{ cm}^2} = \frac{X \text{ cells}}{16.328 \text{ cm}^2}$$

from B. Gonzalez (2020)

PSIS scaffold

Seeding density: 2 million cells per strip

$$2.5 X = 32.656 \text{ million cells}$$

$$X = 13.1 \text{ million cells}$$

$$\text{VEC} = \text{VIC ratio} = 27:34 \text{ from Wang, X. (2018)}$$

$$\text{VEC} + \text{VIC} = 13,100,000$$

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6/13/2022  
Date

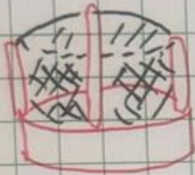
$$\frac{VEC}{VIC} = \frac{27}{34} \Rightarrow VEC = \frac{27}{34} \times VIC$$

$$\frac{27}{34} \times VIC + VIC = 13,100,000$$

$$\frac{61}{34} \times VIC = 13,100,000$$

VIC = 7.3 million } for 1 side of scaffold  
VEC = 5.8 million

1 valve PSIS scaffold



VIC: 14.6 million  
VEC: 11.6 million

} 2 sides



- 6/13/2022: Plated hVEC (P5) and hVIC (P3) in two separate T75 flasks  
 6/14/2022: media change for both VEC & VIC  
 6/20/2022: Seeded hVICs on PSIS  
 6/23/2022: Changed hVEC media and added celltracker dye (red)  
 6/25/2022: Seeded hVECs on the hVICs, and seeded another plate of hVICs alone w/no exposure to hVECs on PSIS  
 7/1/2022: Started PC conditioning  
     hVEC-hVIC static  
     hVEC-hVIC flow  
     hVIC only static  
     hVIC only flow  
 7/8/2022: Collected all conditioned media and placed samples in formalin 10%  
 7/9/2022: Embedded samples in OCT, stored in -80 °C  
 7/25/2022: sectioned w/cryostat at AHC I

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6/13/2022

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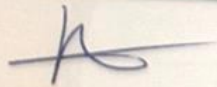
Date \_\_\_\_\_

**PACKING LIST (PURCHASE ORDER # PO-39202022)**

SHIPPER	CONSIGNEE
Jorge Gamiz Innovative Technologies in Biological Systems S.L. Parque Tecnológico de Bizkaia Edificio 502-1ª planta 48.160 Derio-Vizcaya Spain Phone number: +34944005355 E-mail: aaldecocea@innoprot.com Tax ID/VAT number: ESB95481909	Att. Dr. Denise Hsu Florida International University Biomedical Engineering Department 10555 West Flagler Street - Suite EC2600 Miami, FL 33174 USA E-mail: chsu013@fiu.edu Phone Number: 305 348 6717

Nº packages	Nº units	Unit of Measure	Country of origin	Description of goods/Harmonized Tariff
2	1	kit	Spain	Fibroblast Medium Kit - II
	1	kit	Spain	Hu. Valvular Interstitial Cells
MANUFACTURER: INNOPROT (SPAIN)				
Cell Culture Medium for stable cell lines Non-hazardous, non-toxic, non-infectious. For laboratory research only. For invitro research purposes only.				

Date: 07/15/2022

Signature: 

7/21/2022

8/1/2022: Plated 1 frozen vial of hVEC (P1) & hVIC (P1)  
8/2/2022: Changed media on hVIC & hVEC  
8/4/2022: passaged hVEC P1 → P2. Made bioreactor media with DMEM + 10% FBS + 2% P/S + 82 µg/mL AA2P + 2 ng/mL bfgf

**Lonza**

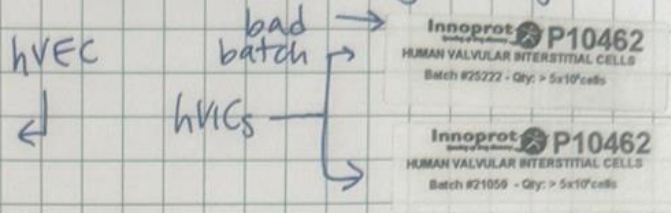
**Store Amps In Liquid Nitrogen Upon Arrival**

Part # 00225975  
Lot # 1F5027  
Qty: 2

Instructions for use:  
[www.lonza.com/cellbioinstructions](http://www.lonza.com/cellbioinstructions)  
Deviation from recommended protocol voids guarantee.

Lonza Walkersville, MD USA 301-898-7025, [www.lonza.com](http://www.lonza.com)

**Lonza** 00521521



hVEC supplements:

<p><b>VEGF</b> CAT. NO.: CC-4114B 0.5 ML ENDOTHELIAL GROWTH FACTOR VASCULAR HUMAN RECOMBINANT LOT NO.: 0000974191 EXP.: 11 FEB 2022 STORE AT -20 °C FOR RESEARCH USE ONLY</p>	<p><b>GA-1000</b> CAT. NO.: CC-4381B 0.5 ML GENTAMICIN SULFATE AMPHOTERICIN-B CELL CULTURE TESTED LOT NO.: 0000974196 EXP.: 16 FEB 2022 STORE AT -20 °C FOR RESEARCH USE ONLY SEE MSDS</p>	<p><b>rhFGF-B</b> CAT. NO.: CC-4113B 2.0 ML HUMAN FIBROBLAST GROWTH FACTOR-B LOT NO.: 0000974190 EXP.: 17 FEB 2022 STORE AT -20 °C FOR RESEARCH USE ONLY</p>
<p><b>AoVEC 41911</b> WARNING: HUMAN SOURCE MATERIAL Human Aortic Valvular Endothelial Cells ≥500,000 CELLS/ML STORE AT -180 °C DATE CRYOPRESERVED: 01 FEB 2021 CAT. #: 00225975 LOT NO.: 1F5027 FOR RESEARCH USE ONLY</p>	<p><b>ASCORBIC ACID</b> CAT. NO.: CC-4115B 0.5 ML IN AQUEOUS SOLUTION CELL CULTURE TESTED LOT NO.: 0000974194 EXP.: 15 FEB 2022 STORE AT -20 °C FOR RESEARCH USE ONLY</p>	<p><b>R<sup>3</sup>-IGF-1</b> CAT. NO.: CC-4115B 0.5 ML RECOMB. LONG R INSULIN-LIKE GROWTH FACTOR-1 IN AQUEOUS SOLUTION CELL CULTURE TESTED LOT NO.: 0000974193 EXP.: 26 FEB 2022 STORE AT -20 °C FOR RESEARCH USE ONLY</p>
<p><b>HYDROCORTISONE</b> CAT. NO.: CC-4112B 0.2 ML WARNING: R11 HIGHLY FLAMMABLE CELL CULTURE TESTED LOT NO.: 0000974189 EXP.: 10 FEB 2022 STORE AT -20 °C FOR RESEARCH USE ONLY</p>	<p><b>rhEGF</b> CAT. NO.: CC-4317B 0.5 ML EPIDERMAL GROWTH FACTOR HUMAN, RECOMB. IN A BUFFERED BSA SALINE SOLUTION LOT NO.: 0000974195 EXP.: 05 MAR 2022 STORE AT -20 °C FOR RESEARCH USE ONLY</p>	<p><b>FBS</b> CAT. NO.: CC-4102B 25 ML FETAL BOWINE SERUM CELL CULTURE TESTED LOT NO.: 0000974180 EXP.: 08 MAR 2025 STORE AT -20 °C FOR RESEARCH USE ONLY</p>

**Lonza**

Clonetics<sup>®</sup>  
**EGM<sup>2</sup> MV SingleQuots<sup>®</sup>**

Catalog No: CC-4147  
Lot No: 0000974197  
Expiration Date: 10 FEB 2022

Contains: Date Added:  
CC-4192B FBS, 25 ML  
CC-4112B HYDROCORTISONE, 0.2 ML  
CC-4113B rhFGF-B, 2 ML  
CC-4114B VEGF, 0.5 ML  
CC-4115B R<sup>3</sup>-IGF-1, 0.5 ML  
CC-4116B ASCORBIC ACID, 0.5 ML  
CC-4317B rhEGF, 0.5 ML  
CC-4381B GA-1000, 0.5 ML

Store at -20 °C  
For Research Use Only. Not for use in diagnostic procedures.

Instructions for use:  
[www.lonza.com](http://www.lonza.com)

Lonza Walkersville, MD USA 301-898-7025 [www.lonza.com](http://www.lonza.com)

**Lonza** 0025

hVIC supplements:

<p><b>Innoprot FBS</b> Qty: 25 ml - Exp: 05/2025 Sterile Filtered Storage: 2-8 °C</p>	<p><b>Innoprot P/S SOLUTION</b> 100x - Qty: 5 ml - Lot: 0000621 made 100% in-house Storage: 2-8 °C - Exp: 12/22</p>	<p><b>Innoprot FGS-2</b> Qty: 5 ml - Exp: 10/2023 For Research use only Storage: 2-8 °C</p>
---	---	---

Bioreactor media:  
bfgf: CORNING  
REF 354060  
Fibroblast Growth Factor,  
Basic Human, Recombinant  
Lot # 2017006

8/6/2022: Passaged hVIC P1 → P2 & hVEC P2 → P3. Plated 3 coverslips for Dani  
8/9/2022: Passaged hVIC P2 → P3 & hVEC P3 → P4. Plated 3 coverslips for Dani  
8/11/2022: Froze 1 T75 hVICs in 5 vials.  
8/12/2022: Seeded hVICs onto PSIS scaffolds (at P3) in BR media.  
Seeding density: 2.5 cm<sup>2</sup>: 2 million cells  
8/16/2022: Seeded hVECS onto PSIS scaffolds

Continued on Page

Read and Understood By

Signed

Date

Signed

Date

8/1/2022

## Certificate of Analysis

Florida International University  
 Attn: Hutcheson/Denise/Claudia  
 Engineering Ctr / EC 02610  
 10555 West Flagler Street  
 Miami FL 33174

**Despatch Date:** 01-Aug-2022  
**Customer Order:** FIU01-0000244627  
**Delivery:** 72518691  
**Sales Order:** 33857732

**Product Name:** hAoVEC-Aortic Valve Endothelial Cells  
**Material Number:** 00225975  
**Batch No:** 1F5339  
**Quantity:** 1.000 AMP  
**Manufacturing Date:** 28-Feb-2022

Test	RESULT	SPECIFICATION		UNIT
		MIN	MAX	
<b>DONOR INFORMATION</b>				
Age	56			
Sex	FEMALE			
<b>VIRUS TESTING</b>				
HIV	Not Detected			
Hepatitis B	Not Detected			
Hepatitis C	Not Detected			
<b>SAFETY TESTING</b>				
Sterility Test	Negative	Negative		
Mycoplasma	Negative	Negative		
<b>CELL STRAIN CALCULATIONS</b>				
Viability	83			
%				Target: >= 70%
Cell Count (cells/amp)	1139400	>= 5x10E5 cells/vial		
Seeding Efficiency	64	For Information Only		9999999 %
Doubling Time	19	For Information Only		9999999 hrs
<b>CELL STAINING</b>				
Factor VIII Expression	27	For Information Only		9999999 %

This lot has been reviewed by Quality Assurance in compliance with requirements of Lonza's Quality System. This document was generated from a validated Part 11-compliant electronic system and thus handwritten signatures are not required.

For Technical Assistance, call 1-800-521-0390  
 signed

Date

Lonza  
 523 Davis Drive Suite 400B  
 Morrisville, NC, 27560

Signed

Date



0072518691

## Packing List



FIU01-0000244

627

<b>Shipping Point:</b> Walkersville 21793-0127	<b>Shipping Terms:</b> CPT - Carriage paid to Incoterms2020
<b>Shipping Point:</b> 21793-0127Walkersville	
<b>Delivery Date:</b> 02-Aug-2022	
<b>Freight Carrier:</b> FEDEX	<b>Delivery:</b> 0072518691
<b>Freight Mode:</b> LBS Overnight Air	
<b>Route:</b> ZLAL1-LBS Overnight Air	

**Customer No.: 6023094**

**Ship to:**

Florida International University  
 Attn: Hutcheson/Denise/Claudia  
 Engineering Ctr / EC 02610  
 10555 West Flagler Street  
 MIAMI FL 33174  
 USA

**Sold to:**

Florida International University  
 10555 West Flagler Street  
 MIAMI FL 33174  
 USA

**Bill to:**

Florida International University  
 Accounts Payable  
 11200 South West 8th Street  
 MIAMI FL 33199  
 USA

Order No.	Order Date	Customer Order No.	Customer Contact
33857732	20-Jul-2022	FIU01-0000244627	Donald Corbitt - 305 348 1243

Line Item	Product Code/Description	Order Qty	Ship Qty	UOM	Lot No./Ser.Nr.	Expiration date	Temperature	Storage Conditions
009	REFRIG REFRIGERATION	1.000	1.000	EA			-20°C	
010	00225975 hAoVEC-Aortic Valve Endothelial Cells	1	1	AMP	1F5339		-180°C	
020	CC-3202 EGM-2 MV BulletKit (CC-3156 & CC-4147)	1.000	1.000	KT			2 to 8 °C	
030	CC-3156 EBM-2 Basal Medium 500 ml	1.000	1.000	BOT	0001111882	18-Apr-2023	2 to 8 °C	
040	CC-4147 EGM-2 MV SingleQuot Kit Suppl. & Growth Factors	1.000	1.000	KT	0001107616	04-Apr-2023	-20°C	

**Delivery Instructions:**



- 8/21/2022: started bioreactor conditioning in Procalcific Bioreactor media
- 8/28/2022: Terminated bioreactor. Placed samples in formalin and stored in 4°C  
 IP: Sigma-aldrich  
 I1643-100 UN  
 Pyrophosphatase, inorganic, from baker's yeast  
 powder  $\geq 500$  units/mg protein (E 1%/280)  
 Stored conditioned media in -80°C
- 8/29/2022: Conducted *in vitro* hydrodynamic tests on conditioned valves.  
 Mechanical: control (26mm)  
 Bioprosthetic: control (27mm)  
 Static PC (26mm) PSIS seeded w/VIC & VEC in Static PC media  
 Bioreactor PC (26mm) PSIS seeded w/VIC & VEC in Bioreactor PC media  
 Raw PSIS  
 Embedded valve tissues after hydrodynamic testing in OCT
- 8/30/2022: Cryostat sectioning @ AHC1  
 Cut thickness: 16  $\mu$ m
- 9/1/2022: ARS staining & BOSE mechanical testing of valve tissues
- 9/2/2022: Imaged ARS stains
- 9/3/2022: Immunostain w/ CD31 &  $\alpha$ SMA antibodies  
 BSA: Cytiva HyClone Laboratories  
 Cat # SH30574.01  
 Lot # AF29530033B  
 Exp: Apr. 14, 2023  
 Primary antibody: Rabbit anti CD31 (green)  
 Prod # PA5-14372  
 Lot # SH2420722B  
 Mouse anti  $\alpha$ SMA (red)  
 Invitrogen eBioscience  
 REF 14-9760-82  
 Lot # 2288516  
 Secondary antibody: ab150108  
 Donkey pAb to Ms IgG  
 Alexa Fluor 594 (red)  
 lot: GR3235186-2  
 ab150073  
 Donkey pAb to Rabbit IgG  
 Alexa Fluor 488 (green)  
 Lot: GR3273045-1

Continued on Page \_\_\_\_\_

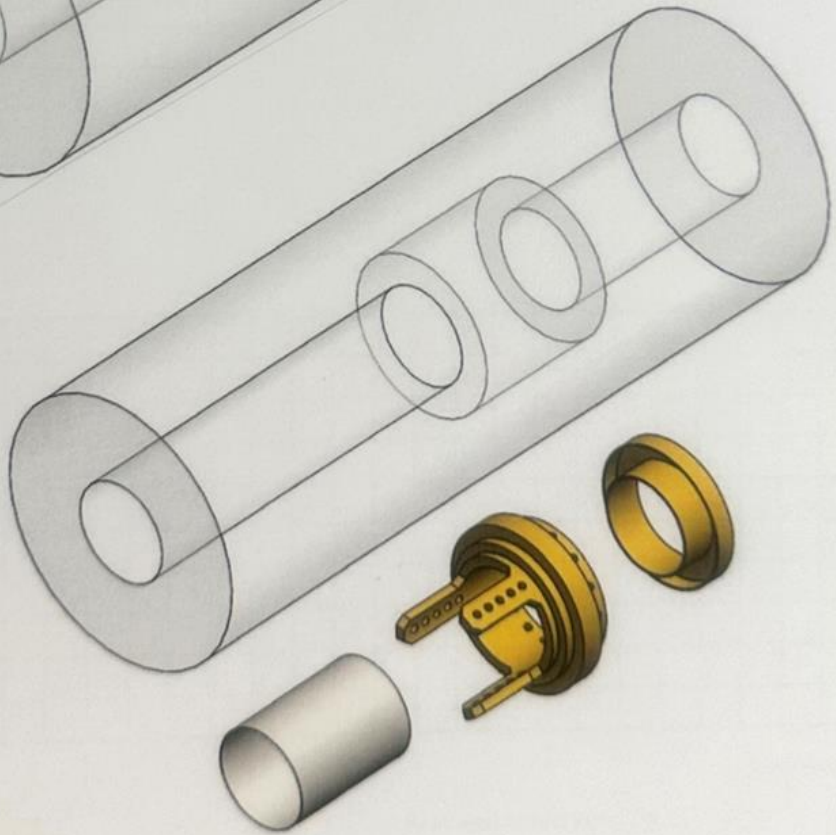
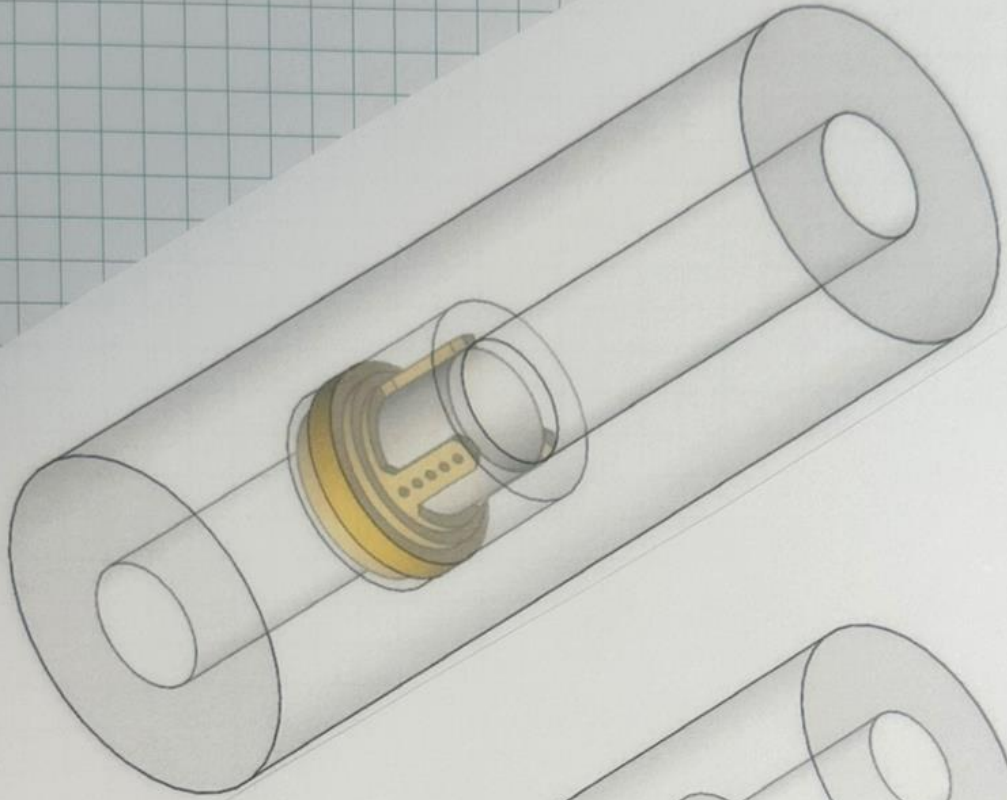
Read and Understood By \_\_\_\_\_

Signed \_\_\_\_\_

Date \_\_\_\_\_

Signed \_\_\_\_\_

8/21/2022  
Date



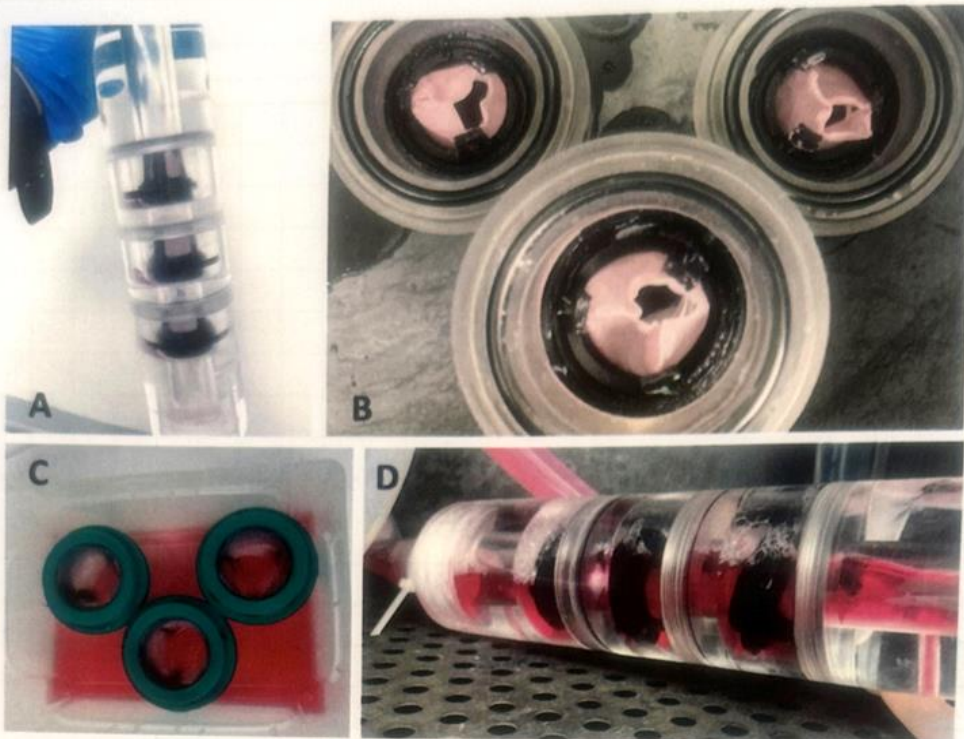


Figure 24. (A) Drained bioreactor with three conditioned PSIS valves. Each valve was seeded with VECs and VICs and conditioned in PC media under 0.50 OSI flow environment. (B) Removal of conditioned valves from bioreactor chambers. (C) Three PSIS valves, each seeded with VECs and VICs and conditioned in static PC media. (D) Bioreactor conditioning of PSIS valves with PC media.

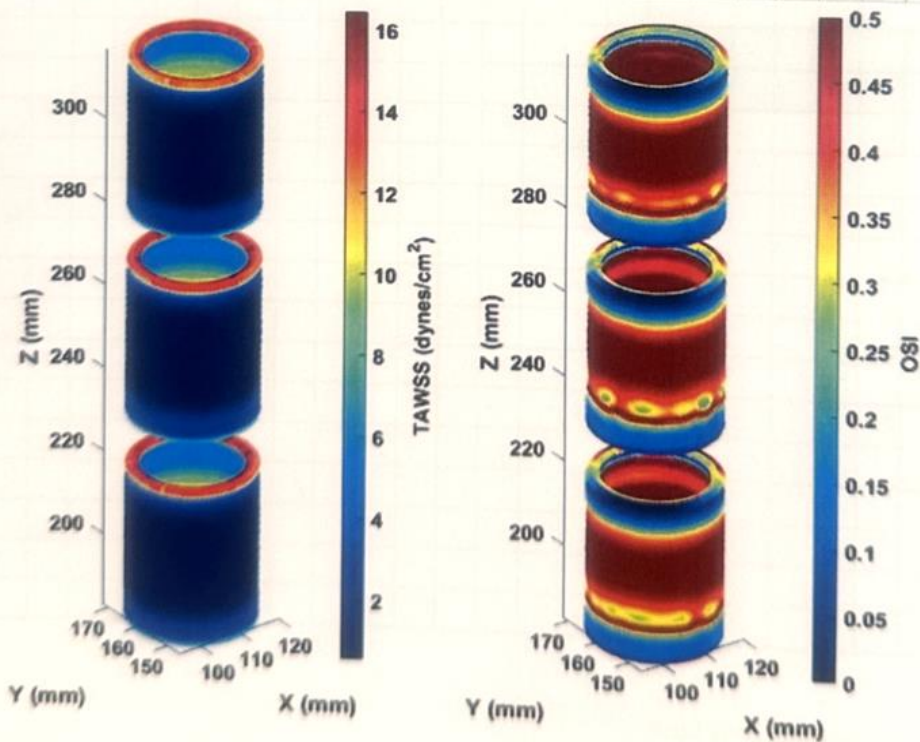
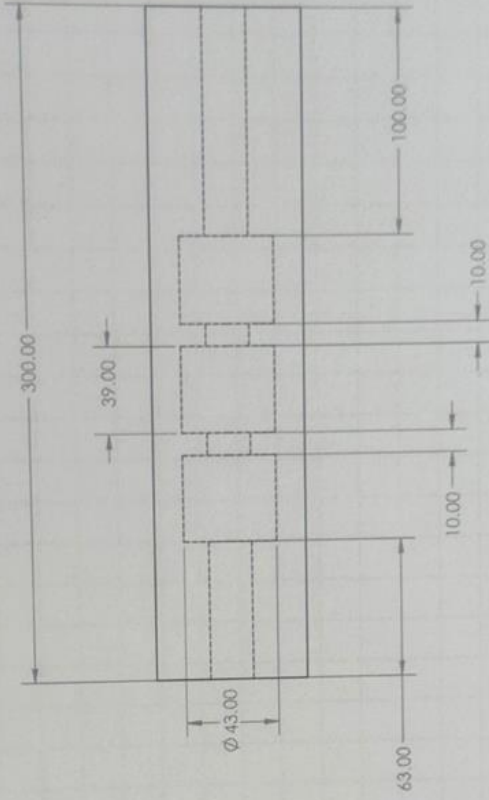
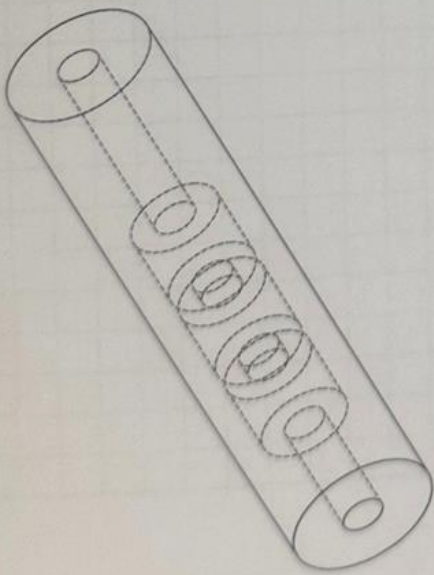


Figure 28. CFD simulation results of wall shear stress and OSI ranges on conditioned valve surfaces



ALL DIMENSIONS GIVEN UNLESS SPECIFIED OTHERWISE ARE IN MILLIMETERS  
UNLESS SPECIFIED OTHERWISE ALL DIMENSIONS ARE IN MILLIMETERS  
TOLERANCES:  
FRACTIONS  
DECIMALS  
ANGLES

DESIGN	NAME	SIGNATURE	DATE

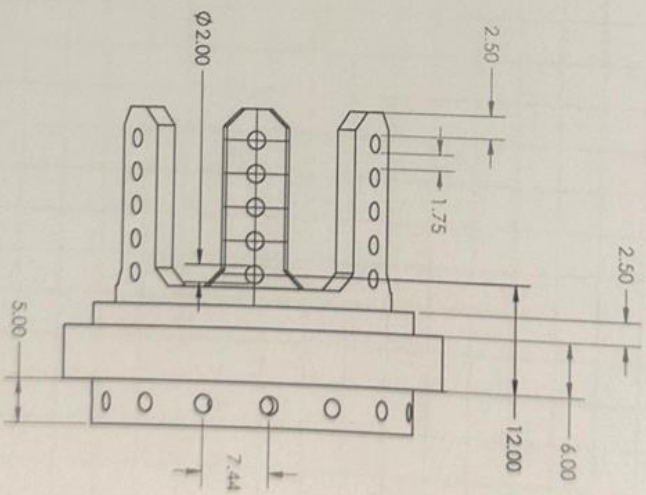
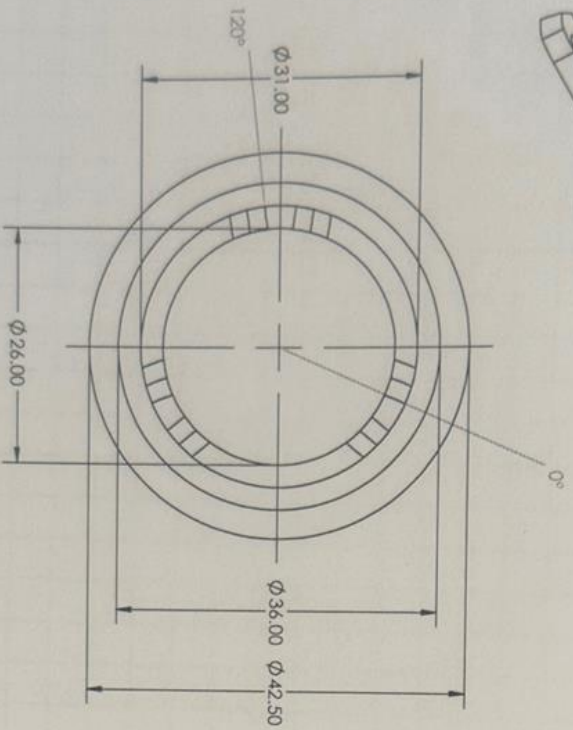
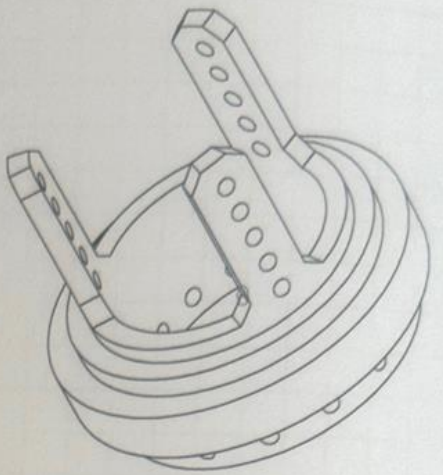
CHECKER AND  
REVISOR AND  
MATERIAL

DO NOT SCALE DRAWING

REGION

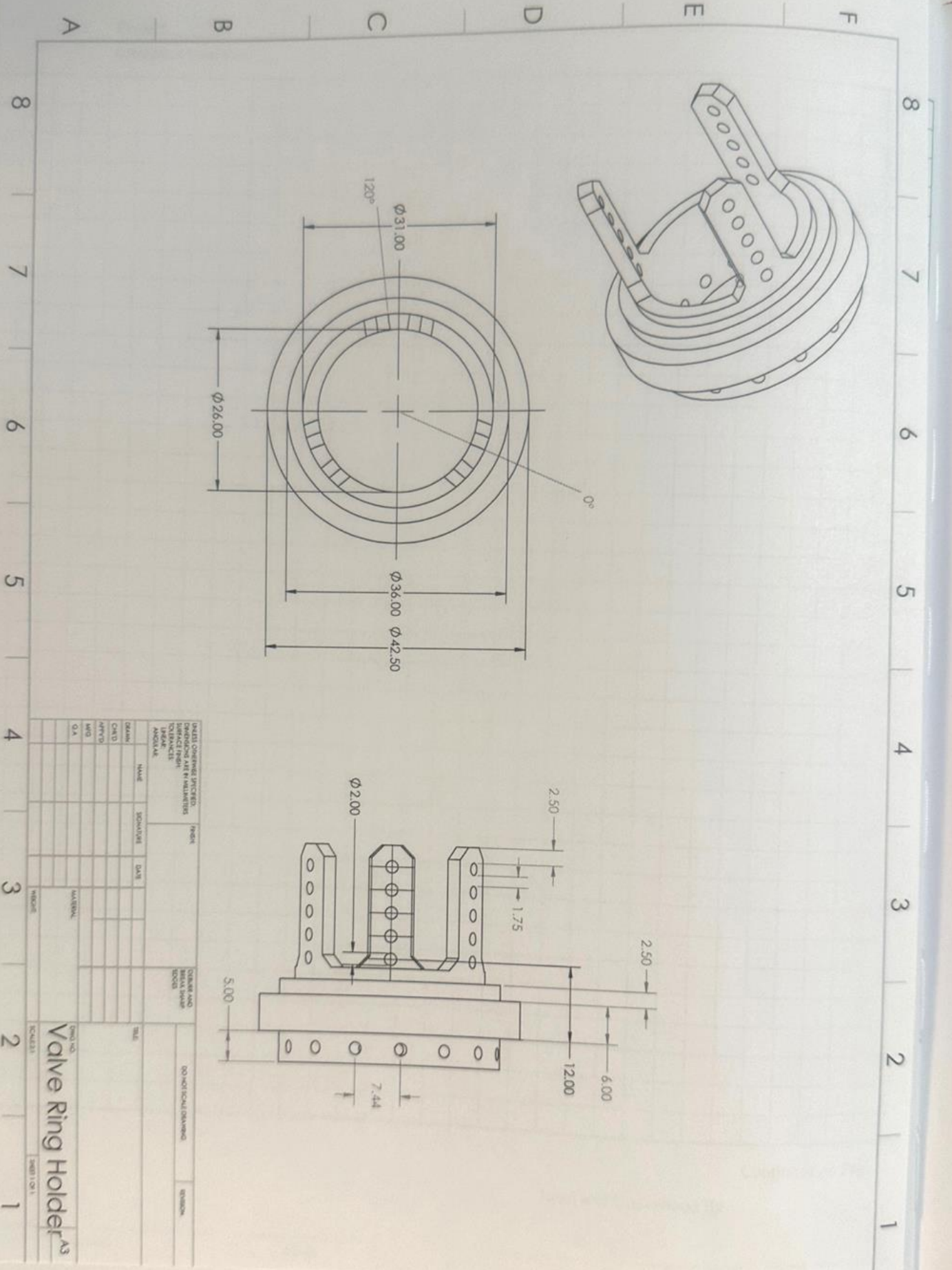
PROJECT NO.  
**Bioreactor Chamber**  
SCALE 1:1  
SHEET 1 OF 1

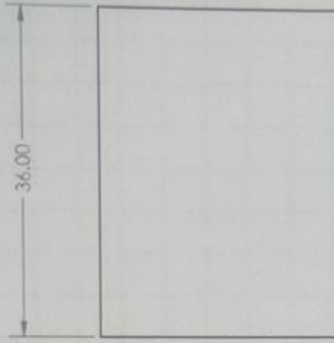
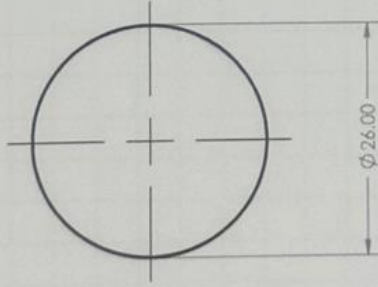
A3



UNITS CONVERSIONS				DATE		DRAWN AND CHECKED		REVISION	
INCHES	MILLIMETERS	FEET	METERS	NAME	DATE	NAME	DATE	NO.	DESCRIPTION
1/8"	3.175	0'	0.000						
1/4"	6.350	0'	0.000						
3/8"	9.525	0'	0.000						
1/2"	12.700	0'	0.000						
5/8"	15.875	0'	0.000						
3/4"	19.050	0'	0.000						
7/8"	22.225	0'	0.000						
1"	25.400	0'	0.000						

DATE	SCALE	TITLE
2023/03/15	1:1	Valve Ring Holder





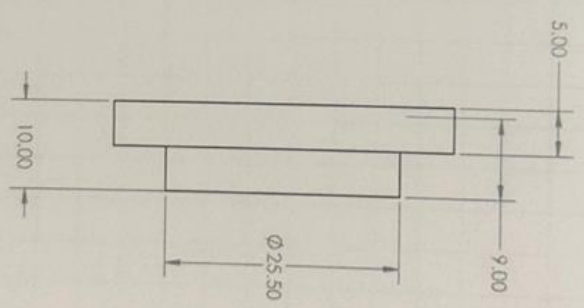
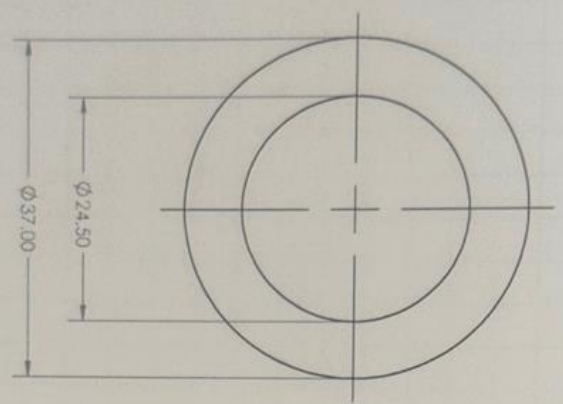
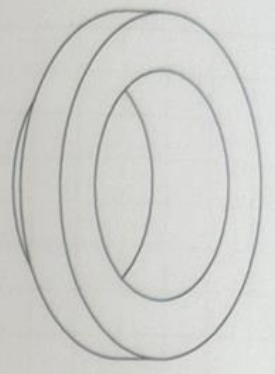
UNLESS OTHERWISE SPECIFIED: DIMENSIONS ARE IN MILLIMETERS SURFACE FINISH TOLERANCES UNLESS OTHERWISE SPECIFIED		FINISH		DESIGN AND DRAWING REVISIONS		DO NOT SCALE DRAWING		REVISION	
DATE	BY	SIGNATURE	DATE	NO.	DESCRIPTION	BY	DATE	NO.	DESCRIPTION

ENGINEER  
Cylindrical PSIS Valve

A3

SCALE: 1:1 SHEET 1 OF 1

8 7 6 5 4 3 2 1



ALL DIMENSIONS UNLESS SPECIFIED  
 ARE IN MILLIMETERS  
 SURFACE FINISH  
 UNLESS SPECIFIED  
 AS PER  
 APPROPRIATE  
 STANDARDS

NO.	REVISION	DATE	BY	APPROVED BY
1				
2				
3				
4				

DATE	NAME	FUNCTION	DATE	REVISION

DRN NO.     TITLE  
 10431     Valve Ring Cap  
 33  
 A3  
 A

8 7 6 5 4 3 2 1

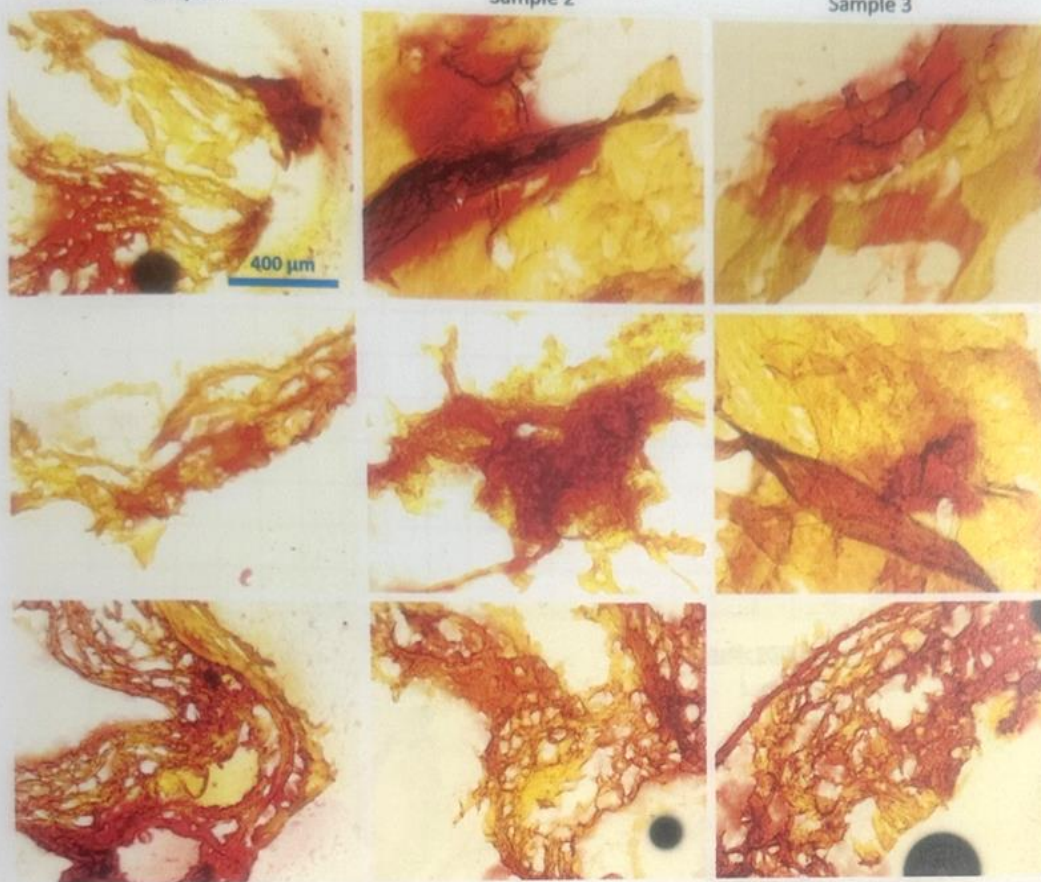
F E D C B A

**Bioreactor PSIS Valves**

Sample 1

Sample 2

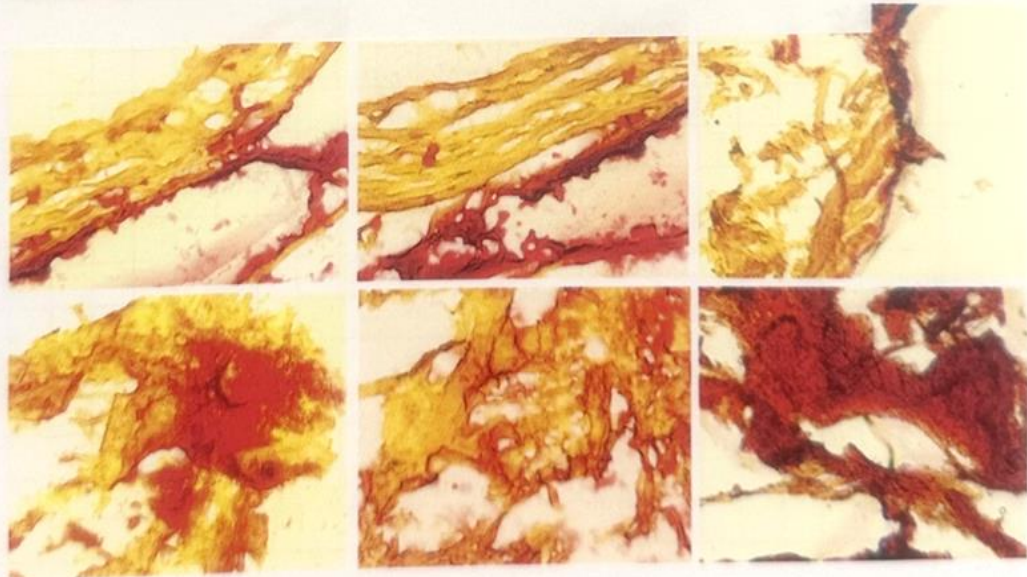
Sample 3



e3



*Figure 36. ARS images of bioreactor-conditioned valves. VECs and VICs were seeded in PSIS and conditioned at 0.50 OSI with PC media for 7 days.*



*Figure 38. ARS images of statically conditioned valves. VECs and VICs were seeded in PSIS and placed in static environment with PC media for 7 days.*

Signed

Date

Signed

Date

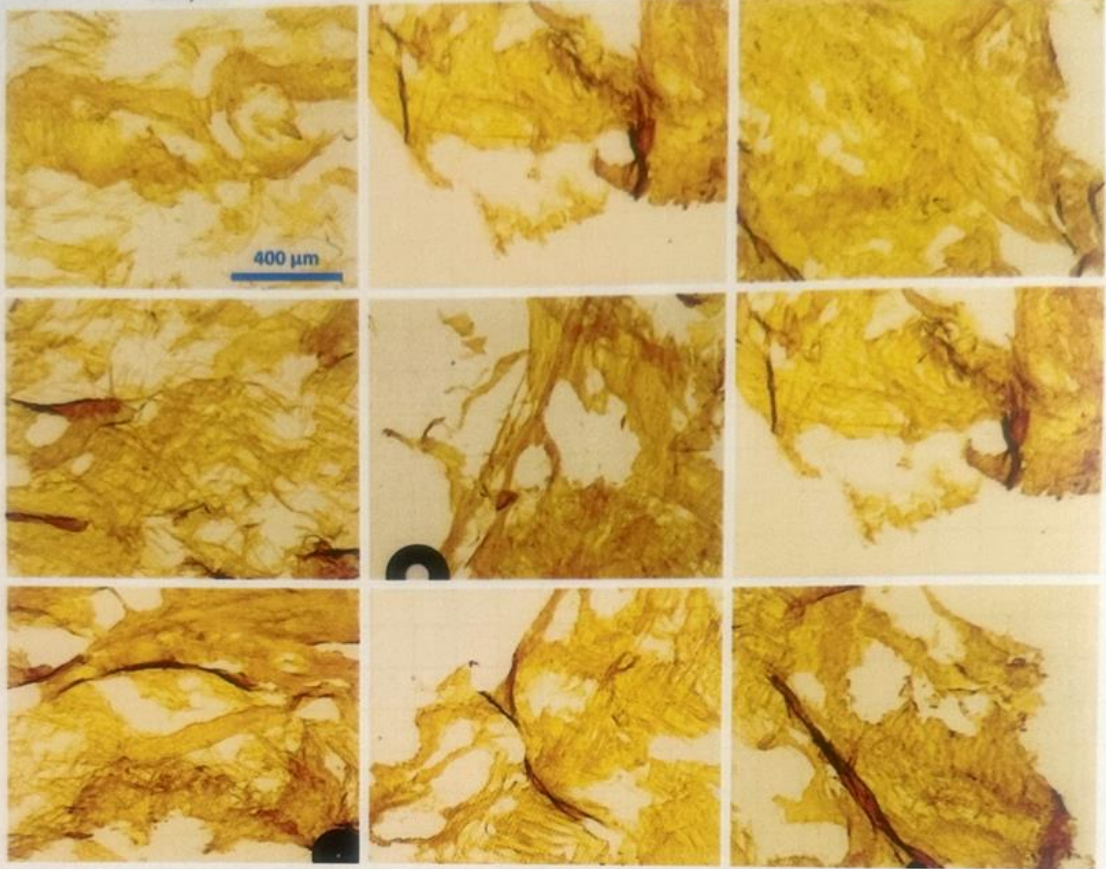


Raw PSIS

Sample 1

Sample 2

Sample 3



*Figure 37. ARS images of raw PSIS.*

Continued on Page

Read and Understood By

Signed

Date

Signed

Date

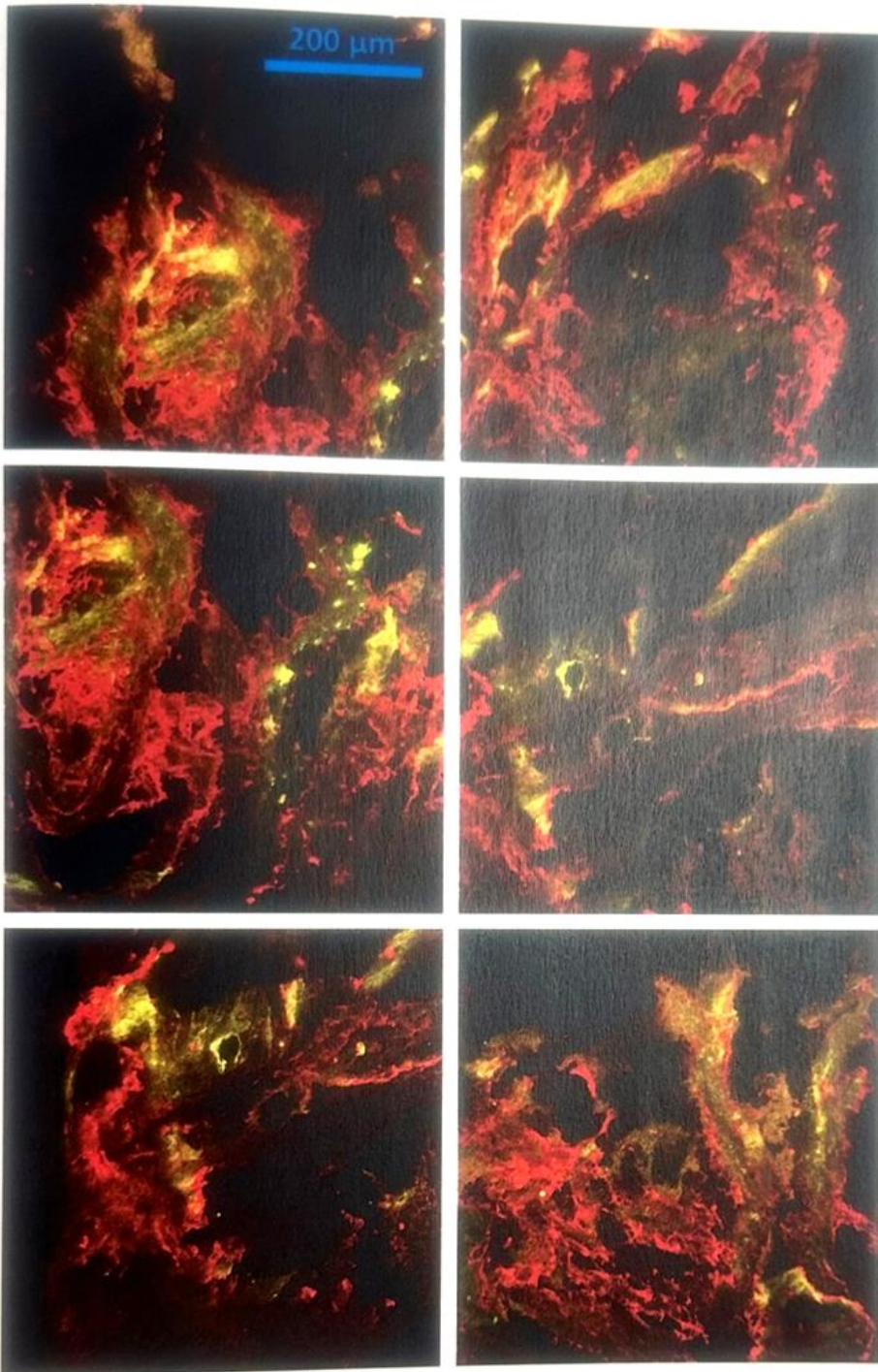


Figure 29. CD31 (green) and  $\alpha$ SMA (red) immunofluorescent stains after conditioning in bioreactor with co-culture of VECs and VICs

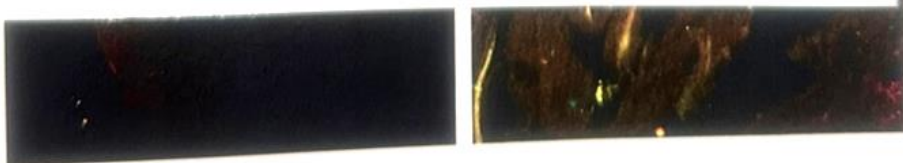


Figure 30. CD31 (green) and  $\alpha$ SMA (red) immunofluorescent stains after conditioning in static with co-culture of VECs and VICs



## Signosis

Innovative Plate Assay Solutions

### Product Information

#### Customized Human Cytokine ELSA Strip (Cus-EA-1001)

#### Product

Customized Human Cytokine ELISA Strip is a sandwich assay for profiling different cytokines in samples. This product is intended for research use only.

#### Components

The product contains all the materials and reagents required for different samples in a 12 X 8 - well assay format.

**\*Please centrifuge all small components before opening\***

- 12 strips, coated with 8 different anti-human cytokine antibodies:**  
Store at 4°C until required  
Ready to use
- Biotin-labeled detection antibody mixture against 8 different human cytokines (200µl):**  
Store in -20°C frost-free freezer until required  
Dilute with 1X Diluent Buffer at 1:50 just before use
- Streptavidin conjugated to horseradish peroxidase (50 µl):**  
Store at 4°C until required  
Dilute with 1X Diluent Buffer at 1:200 just before use
- 1X Diluent Buffer (40ml):**  
Store at 4°C until required  
Ready to use
- 5X Assay Wash Buffer (40ml):**  
Store at 4°C until required  
Dilute 40ml of 5X Assay Wash Buffer with 160 ml of dH<sub>2</sub>O before use
- Substrate (10 ml):**  
Store at 4°C until required  
Ready to use
- Stop Solution (2N H<sub>2</sub>SO<sub>4</sub>, 5ml):**  
Store at 4°C until required  
Ready to use  
**\*\*Caution: It is a strong acid substance. Therefore, be careful not to contact your skin and clothes with Stop solution and pay attention to the disposal of Stop solution.\*\***

#### Shelf Life

The shelf life of this product is 6 months. Use it before expiration.

#### Contacting Signosis

For technical questions, contact our technical support group by telephone at 1-408-747-0771 or by email at [support@signosisinc.com](mailto:support@signosisinc.com)



**Signosis**  
Innovative Plate Assay

## Customized Human Cytokine ELISA Strip

Catalog # Cus-EA-1001\_inv#005289

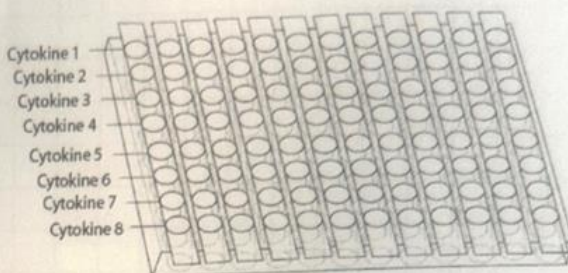
(For Research Use Only)

### Introduction

Cytokines are essential molecules that have crucial roles in many biological functions including viral infection, inflammation, immunity, and hematopoiesis. Cytokines are produced by a variety of cell types in response to different stimuli. In addition, the expression of cytokine genes appears to be regulated by complex mechanisms. Expression of one cytokine gene could be regulated by other cytokines. Dysregulation of cytokine gene expression may be caused by chromosomal alterations or by infection of viruses that induce activation or inactivation of the expression machinery. Therefore, profiling of these cytokines is critical in understanding these biological functions. Signosis' Customized Cytokine ELISA Strip quantitatively profiles and measures 8 human cytokines: TGF $\beta$ , VEGF, TNF $\alpha$ , IL-1 $\beta$ , IL-6, IL-8, MCP-1, and GM-CSF. The difference of these proteins between samples can be determined through data comparison.

### Principle of the assay

In each well of the strip, a primary antibody against a specific cytokine is coated and 8 wells of the strip are coated with 8 different antibodies. The test sample is allowed to react simultaneously with pairs of two antibodies, resulting in the cytokines being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentrations of the cytokines are directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.



Incubate with Detection Antibody Mixture

Incubate with HRP-Streptavidin

OD450 Reading

Diagram of Cytokine ELISA Strip

### Materials provided with the kit

Component	Qty	Store at
96-Well 12 strip Plate coated with 8 different antibodies against customized human cytokines	1	4°C
Biotin labeled antibody mixture against 8 different human cytokines	200 $\mu$ L	-20°C
Streptavidin-HRP conjugate	50 $\mu$ L	4°C
1X Diluent buffer	40 mL	4°C
5X Assay wash buffer	40 mL	4°C
Substrate	10 mL	4°C
Stop solution	5 mL	4°C

**Reagent preparation before starting experiment**

- Dilute the 5x Assay wash buffer to 1x buffer:  
- 40 ml 5x Assay wash buffer  
- 160 ml ddH<sub>2</sub>O
- Dilute 50 times of biotin labeled antibody mixture with 1X Diluent buffer.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer.

**Sample preparation before starting experiment**

- For **cell culture medium samples**, add 100  $\mu$ l directly to the well.
- For **cell lysate samples**, use cell lysis buffer (Catalog# EA-0001). Follow protocol in Cell Lysate Buffer User Manual.
- For **serum or plasma samples**, we recommend a 1:10 dilution with 1X diluent buffer, for example, add 80ul sample in 720 ul 1X diluent buffer. When serum-containing conditional media is required, be sure to use serum as control.

**Recommendation**

- If you would like to quantitatively measure the cytokines in the samples, you can make standard curves through a series of 2-fold dilutions of protein standards. Protein standards can be purchased separately from Signosis.

**Assay procedure**

1. Take the desired strips from the plate. Make sure the rest of strips are well sealed.
2. Standard curve (optional):  
If protein standard curve is desired, 4-5 wells for a cytokine may be used to make Standard curve.
3. Sample assay:  
Apply each sample on the well, 100ul per well and incubate for 1-2 hour at room temperature with gentle shaking.
4. Aspirate each well and wash by adding 200  $\mu$ l of 1X assay wash buffer. Repeat the process three times for a total of three washes. Completely remove liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
5. Add 100  $\mu$ l of diluted biotin-labeled antibody mixture to each well and incubate for 1 hour at room temperature with gentle shaking.
6. Repeat the aspiration/wash as in step 4.
7. Add 100  $\mu$ l of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
8. Repeat the aspiration/wash as in step 4.
9. Add 100  $\mu$ l substrate to each well and incubate for 10-30 minutes.

**Note: Substrate incubation time may vary due to different antibodies reactivity. Stronger signals (Strong blue color) could be stopped early after 5 minutes. Weaker signals should be incubated for 10-30 minutes.**

9. Add 50  $\mu$ l of Stop solution to each well. The color in the wells should change from blue to yellow.
10. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

**Customized Human 8 Cytokine ELISA Strip Diagram**

	1	2	3	4	5	6	7	8	9	10	11	12
A	TGF $\beta$	TGF $\beta$	TGF $\beta$	TGF $\beta$	TGF $\beta$	TGF $\beta$	TGF $\beta$	TGF $\beta$	TGF $\beta$	TGF $\beta$	TGF $\beta$	TGF $\beta$
B	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF	VEGF
C	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$	TNF $\alpha$
D	IL-1 $\beta$	IL-1 $\beta$	IL-1 $\beta$	IL-1 $\beta$	IL-1 $\beta$	IL-1 $\beta$	IL-1 $\beta$	IL-1 $\beta$	IL-1 $\beta$	IL-1 $\beta$	IL-1 $\beta$	IL-1 $\beta$
E	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6	IL-6
F	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8	IL-8
G	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1	MCP-1
H	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF	GM-CSF

CARDIAC CELL SYSTEM INNOPROFILE™  
HUMAN CARDIAC VALVULAR INTERSTITIAL CELLS



<b>Product Type:</b>	Cryo-preserved Valvular Interstitial Cells
<b>Catalog Number:</b>	P10462
<b>Source:</b>	Human Heart Valves
<b>Number of Cells:</b>	5 x 10 <sup>5</sup> Cells / vial (1ml)
<b>Storage:</b>	Liquid Nitrogen

Human Valvular Interstitial Cells (HVIC) provided by Innoprot have been derived from heart valves that are explanted in culture. Human valvular interstitial cells are from a single donor. They are cryopreserved at primary culture and can be cultured and propagated at least 10 population doublings in the conditions provided by Innoprot.

These cells are positive for smooth muscle actin. These cells enable researchers to study the role of cardiac valves in vitro. Human valvular interstitial cells may be used for various types of valve replacement, stimulus contraction and transplantation studies into normal or diseased systems. In addition, they may be used for tissue engineering applications.

**Product Use**

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for use in vitro diagnostic or clinical procedures

**Recommended Medium**

- Fibroblast Medium II Kit  
(Reference: P60166)



**Product Characterization**

Immunofluorescent method

- $\alpha$ -smooth muscle actin
- Vimentin

The cells test negative for HIV-1, HIV-II, HBV, HCV, mycoplasma, bacteria, yeast and fungi

7/21/2022



## INSTRUCTIONS FOR CULTURING CELLS

**IMPORTANT:** Cryopreserved cells are very delicate. Thaw the vial in a 37 °C waterbath and return them to culture as quickly as possible with minimal handling!

### Set up culture after receiving the order:

1. Prepare a poly-L-lysine coated flask (2  $\mu\text{g}/\text{cm}^2$ , T-75 flask is recommended). Add 10 ml of sterile water to a T-75 flask and then add 150  $\mu\text{l}$  of poly-L-lysine stock solution (1 mg/ml, Innoprot cat. no. PLL). Leave the flask in incubator overnight (minimum one hour at 37°C incubator).
2. Prepare complete medium: decontaminate the external surfaces of medium and medium supplements with 70% ethanol and transfer them to sterile field. Aseptically open each supplement tube and add them to the basal medium with a pipette. Rinse each tube with medium to recover the entire volume.
3. Rinse the poly-L-lysine coated flask with sterile water twice and add 20 ml of complete medium to the flask. Leave the flask in the hood and go to thaw the cells.
4. Place the vial in a 37°C waterbath, hold and rotate the vial gently until the contents are completely thawed. Remove the vial from the waterbath immediately, wipe it dry, rinse the vial with 70% ethanol and transfer it to a sterile field. Remove the cap, being careful not to touch the interior threads with fingers. Using 1 ml eppendorf pipette gently resuspend the contents of the vial.
5. Dispense the contents of the vial into the equilibrated, poly-L-lysine coated culture vessels. A seeding density of 5,000 cells/cm<sup>2</sup> is recommended.

Note: Dilution and centrifugation of cells after thawing are not recommended since these actions are more harmful to the cells than the effect of DMSO residue in the culture. It is also important that fibroblasts are plated in poly-L-lysine coated culture vessels that promote cell attachment.

6. Replace the cap or cover, and gently rock the vessel to distribute the cells evenly. Loosen cap if necessary to permit gas exchange.
7. Return the culture vessels to the incubator.
8. For best result, do not disturb the culture for at least 16 hours after the culture has been initiated. Change the growth medium the next day to remove the residual DMSO and unattached cells, then every other day thereafter.

### Maintenance of Culture:

1. Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells..
2. Change the medium every three days thereafter, until the culture is approximately 70% confluent.
3. Once the culture reaches 70% confluence, change medium every other day until the culture is approximately 90% confluent.

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**Subculture:**

1. Subculture the cells when they are over 90% confluent.
2. Prepare poly-L-lysine coated flasks (2  $\mu\text{g}/\text{cm}^2$ ) one day before subculture.
3. Warm medium, trypsin/EDTA solution (T/E, cat. no. 0103), trypsin neutralization solution (TNS, cat. no. 0113), and DPBS to room temperature. We do not recommend warming the reagents and medium at 37°C waterbath prior to use.
4. Rinse the cells with DPBS.
5. Add 8 ml of DPBS first and then 2 ml of trypsin/EDTA solution into flask (in the case of T-75 flask); gently rock the flask to make sure cells are covered by trypsin/EDTA solution; incubate the flask at 37°C incubator for 1 to 3 minutes or until cells are completely rounded up (monitored with inverted microscope). During incubation, prepare a 50 ml conical centrifuge tube with 5 ml of fetal bovine serum; transfer trypsin/EDTA solution from the flask to the 50 ml centrifuge tube (a few percent of cells may detached); continue incubate the flask at 37°C for 1 minutes (no solution in the flask at this moment); at the end of trypsinisation, one hand hold one side of flask and the other hand gently tap the other side of the flask to detach cells from attachment; check the flask under inverted microscope to make sure all cells are detached, add 5 ml of trypsin neutralization solution to the flask and transfer detached cells to the 50 ml centrifuge tube; add another 5 ml of TNS to harvest the residue cells and transfer it to the 50 ml centrifuge tube. Examine the flask under inverted microscope to make sure the cell harvesting is successful by looking at the number of cells left behind. There should be less than 5%.

Note: DPBS, trypsin/EDTA solution & trypsin neutralization solution are included in the "Primary Cells Detach Kit provided by Innoprot (Cat. N° P60305).

6. Centrifuge the 50 ml centrifuge tube (harvested cell suspension) at 1000 rpm (Beckman Coulter Allegra 6R centrifuge or similar) for 5 min; re-suspend cells in growth medium.
7. Count cells and plate cells in a new, poly-L-lysine coated flask with cell density as recommended.

**Caution:** Handling human derived products is potentially biohazardous. Although each cell strain testes negative for HIV, HBV and HCV DNA, diagnostic tests are not necessarily 100% accurate, therefore, proper precautions must be taken to avoid inadvertent exposure. Always wear gloves and safety glasses when working these materials. Never mouth pipette. We recommend following the universal procedures for handling products of human origin as the minimum precaution against contamination [1].

[1]. Grizzle, W. E., and Polt, S. S. (1988) Guidelines to avoid personal contamination by infective agents in research laboratories that use human tissues. *J Tissue Culture Methods*. 11(4).





## FIBROBLAST MEDIUM-2

**Product Type:** Fibroblast Medium-2  
**Catalog Number:** P60108-2

### Product Description

Fibroblast Medium-2 (FM-2) is a complete medium designed for optimal growth of normal human cardiac fibroblasts in vitro. It is a sterile, liquid medium which contains essential and non-essential amino acids, vitamins, organic and inorganic compounds, hormones, growth factors, trace minerals and a low concentration of fetal bovine serum (5%). The medium is HEPES and bicarbonate buffered and has a pH of 7.4 when equilibrated in an incubator with an atmosphere of 5% CO<sub>2</sub>/95% air. The medium is formulated (quantitatively and qualitatively) to provide a defined and optimally balanced nutritional environment that selectively promotes proliferation and growth of normal human cardiac fibroblasts in vitro.

### Components

- 500 ml of Basal Medium
- 25 ml of Fetal Bovine Serum (FBS)
- 5 ml of Fibroblast Growth Supplement 2 (FGS-2)
- 5 ml of penicillin/streptomycin solution (P/S solution)

### Prepare for use

Thaw FGS-2, FBS and P/S solution at 37°C. Gently tilt the FGS tube several times during thawing to help the contents dissolve. **Make sure the contents of the supplement are completely dissolved into solution before adding to the medium.** Rinse the bottle and tubes with 70% ethanol, and then wipe to remove excess. Remove the cap, being careful not to touch the interior threads with fingers. Add FGS, FBS and P/S solution into basal medium in a sterile field, mix well and then the reconstituted medium is ready for use. Since several components of this medium are light-labile, it is recommended that the medium not be exposed to light for lengthy periods of time. If the medium is warmed prior to use, do not exceed 37°C. When stored in the dark at 4°C, the reconstituted medium is stable for one month.

### Caution

If handled improperly, some components of the medium may present a health hazard. Take appropriate precautions when handling it, including the wearing of protective clothing and eyewear. Dispose of properly.

7/21/2022

# PERIODIC TABLE OF THE ELEMENTS

1 <b>H</b> 1.0079	2 <b>He</b> 4.0026																																													
3 <b>Li</b> 6.941	4 <b>Be</b> 9.0122	5 <b>B</b> 10.811	6 <b>C</b> 12.011	7 <b>N</b> 14.007	8 <b>O</b> 15.999	9 <b>F</b> 18.998	10 <b>Ne</b> 20.1797																																							
11 <b>Na</b> 22.989	12 <b>Mg</b> 24.305	13 <b>Al</b> 26.981	14 <b>Si</b> 28.085	15 <b>P</b> 30.974	16 <b>S</b> 32.06	17 <b>Cl</b> 35.453	18 <b>Ar</b> 39.948																																							
19 <b>K</b> 39.098	20 <b>Ca</b> 40.078	21 <b>Sc</b> 44.955	22 <b>Ti</b> 47.887	23 <b>V</b> 50.9415	24 <b>Cr</b> 51.9961	25 <b>Mn</b> 54.938	26 <b>Fe</b> 55.845	27 <b>Co</b> 58.933	28 <b>Ni</b> 58.6934	29 <b>Cu</b> 63.546	30 <b>Zn</b> 65.38	31 <b>Ga</b> 69.723	32 <b>Ge</b> 72.63	33 <b>As</b> 74.921	34 <b>Se</b> 78.971	35 <b>Br</b> 79.904	36 <b>Kr</b> 83.798																													
37 <b>Rb</b> 85.467	38 <b>Sr</b> 87.62	39 <b>Y</b> 88.9058	40 <b>Zr</b> 91.224	41 <b>Nb</b> 92.9063	42 <b>Mo</b> 95.95	43 <b>Tc</b> (98)	44 <b>Ru</b> 101.07	45 <b>Rh</b> 102.90	46 <b>Pd</b> 106.42	47 <b>Ag</b> 107.8682	48 <b>Cd</b> 112.414	49 <b>In</b> 114.818	50 <b>Sn</b> 118.710	51 <b>Sb</b> 121.760	52 <b>Te</b> 127.60	53 <b>I</b> 126.90	54 <b>Xe</b> 131.29																													
55 <b>Cs</b> 132.905	56 <b>Ba</b> 137.327	57-71* <b>La</b>	72 <b>Hf</b> 178.49	73 <b>Ta</b> 180.94	74 <b>W</b> 183.84	75 <b>Re</b> 186.207	76 <b>Os</b> 190.23	77 <b>Ir</b> 192.217	78 <b>Pt</b> 195.084	79 <b>Au</b> 196.96	80 <b>Hg</b> 200.59	81 <b>Tl</b> 204.38	82 <b>Pb</b> 207.2	83 <b>Bi</b> 208.98	84 <b>Po</b> (209)	85 <b>At</b> (210)	86 <b>Rn</b> (222)																													
87 <b>Fr</b> (223)	88 <b>Ra</b> (226)	89-103** <b>Ac</b>	104 <b>Rf</b> (261)	105 <b>Db</b> (262)	106 <b>Sg</b> (263)	107 <b>Bh</b> (264)	108 <b>Hs</b> (265)	109 <b>Mt</b> (266)	110 <b>Ds</b> (267)	111 <b>Rg</b> (268)	112 <b>Cn</b> (269)	113 <b>Uut</b> (270)	114 <b>Ff</b> (271)	115 <b>Uup</b> (272)	116 <b>Lv</b> (273)	117 <b>Uus</b> (274)	118 <b>Uuo</b> (286)																													
																		57 <b>La</b> 138.90	58 <b>Ce</b> 140.116	59 <b>Pr</b> 140.90	60 <b>Nd</b> 144.242	61 <b>Pm</b> (145)	62 <b>Sm</b> 150.36	63 <b>Eu</b> 151.964	64 <b>Gd</b> 157.25	65 <b>Tb</b> 158.92	66 <b>Dy</b> 162.50	67 <b>Ho</b> 164.93	68 <b>Er</b> 167.258	69 <b>Tm</b> 168.93	70 <b>Yb</b> 173.054	71 <b>Lu</b> 174.967														
																		89 <b>Ac</b> (227)	90 <b>Th</b> 232.0377	91 <b>Pa</b> 231.03	92 <b>U</b> 238.02	93 <b>Np</b> (237)	94 <b>Pu</b> (244)	95 <b>Am</b> (243)	96 <b>Cm</b> (247)	97 <b>Bk</b> (247)	98 <b>Cf</b> (251)	99 <b>Es</b> (252)	100 <b>Fm</b> (257)	101 <b>Md</b> (258)	102 <b>No</b> (259)	103 <b>Lr</b> (262)														

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## CONVERSION FACTORS

To Convert From	To	Multiply By
Centimeters	Inches	0.39370079
Cubic Feet	Liters	28.31605
Cu. Ft/min	Liters/sec	0.4719342
Cu.meters/min	Liters/min	999.972
Feet	Centimeters	30.48
Feet/min	Meters/sec	0.00508
Gallons	Liters	3.785306
Gal./sec	Liters/min	227.1183
Horsepower	Btu/hr	2547.16
Horsepower	Watts	746
Inches	Centimeters	2.54
Inches of H <sub>2</sub> O (4°C)	Dynes/sq cm.	2490.82
Inches of H <sub>2</sub> O (4°C)	In. of Hg (32°)	.07355
Kilograms	Pounds	2.2046226
Kilowatts	Btu/hr	3414.43
Liters	Gallons	0.2641794
Meters/sec	Feet/min	196.85039
Milliliters	Ounces	0.03381497
Millimeters	Inches	0.039370079
Millimeters of Hg (0°)	Pounds/sq. in.	0.0193368
Ounces	Liters	0.029572702
Pounds	Kilogram	0.45359237
Sq. Feet	Sq. Meters	0.09290304
Sq. Meters	Sq. Feet	10.763910
Watts	Btu/hr.	3.41443

## PRESSURE/VACUUM CONVERSIONS

micron = Torr	x 1000
Torr = mBar	x .075
psi = Torr	x 0.019
psi = in Hg vac (abs)	x 0.491
mBar = Torr	x 1.33
psi = in Hg vac (abs)	x 33.86
Pascal = Torr	x 133.3

## CONDUCTIVITY CONVERSIONS

μS/cm = μmoh/cm	x 1
mS/cm = μS/cm	x 100
ppm = μS/cm	x 0.5

## CONCENTRATION CONVERSIONS

Molar (M) = $\frac{\text{Moles of solute}}{\text{Liters of solution}}$
Weight % = $\frac{\text{g of solute} \times 100\%}{\text{g of solute} + \text{g of solvent}}$
Volume % = $\frac{\text{Liters of solute} \times 100\%}{\text{Liters of Solution}}$
ppm = $\frac{\text{mg of solute}}{\text{kg of solution}} = \frac{\text{mg of solute}}{\text{Liters of water}}$

## DENSITY CONVERSIONS

Specific Gravity x 1 = g/mL
g/L x 8.345404 = lb/gal
lb/gal x 0.119826 = g/mL

## RELATIVE CENTRIFUGAL FORCE

To calculate RFC in centimeters:  
 $RCF = 0.0000118 \times r \times N^2$

To calculate RFC in inches:  
 $RCF = 28.38 \times (N/1000)^2 \times r$

RCF = Relative Centrifugal Force  
 r = Rotating Radius (cm or inches)  
 N = Rotating Speed (rpm)

## METRIC PREFIXES

Prefix	Abbreviation	Meaning
tera-	T	$\times 10^{12}$
giga-	G	$\times 10^9$
mega-	M	$\times 10^6$
kilo-	k	$\times 10^3$
deci-	d	$\times 10^{-1}$
centi-	c	$\times 10^{-2}$
milli-	m	$\times 10^{-3}$
micro-	μ	$\times 10^{-6}$
nano-	n	$\times 10^{-9}$
pico-	p	$\times 10^{-12}$

## TEMPERATURE SCALES

°C: degree Celcius (centigrade)

°F: degree Fahrenheit

K: Kelvin

	°C	°F	K
Boiling point of water (at 1 atm = 101325 Pa)	100	212	373.15
Freezing point of water (at 1 atm = 101325 Pa)	0	32	273.15
Interval freezing point/boiling point of water (at 1 atm = 101325 Pa)	100	100	180
Triple point of water (solid-liquid-gas equilibrium)	0.01	32.02	273.16

## TEMPERATURE CONVERSIONS

°C = $[(°F - 32)(5/9)]$
°F = $[(°C)(9/5)] + 32$
K = $(°C + 273.15)$
K = $(TK - 273.15)$
°C = $[1.80 * (K - 273.15) + 32] °F$

## GEOMETRIC AREA FORMULAS

A = Area B = Base H = Height  
 R = Radius S = Side π = 3.14159

Triangle =  $B \times H/2$   
 Square =  $S \times S$   
 Rectangle =  $S1 \times S2$   
 Parallelogram =  $B \times H$   
 Regular Pentagon =  $1.720 \times S \times S$   
 Regular Hexagon =  $2.598 \times S \times S$   
 Regular Octagon =  $4.828 \times S \times S$   
 Circle Area =  $\pi \times R^2$   
 Circle Circumference =  $2 \times \pi \times R$   
 Ring =  $\pi \times ((R2 \times R2) - (R1 \times R1))$   
 Ellipse =  $\pi \times R1 \times R2$   
 Sphere Area =  $4 \times \pi \times R^2$   
 Sphere Volume =  $(4/3) \times \pi \times R^3$   
 Cone Volume =  $(1/3) \times \pi \times R^2 \times H$   
 Cylinder Volume =  $\pi \times R^2 \times H$

## OHM LAW RELATIONSHIPS

E = $IR + W/I = \sqrt{WR}$
W = $I^2R = E^2/R = EI$
I = $E/R = W/E = \sqrt{WR}$
R = $E/I = W/I^2 = E^2/W$

For direct current

