USER MANUAL

Culture Plate & Loading Station™

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Rev 9.0

Culturing Cells in a Mechanically Active Environment™

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TABLE OF CONTENTS

Introduction 1
Principle of Loading Stations™ and Equibiaxial Strain 1
Principle of Loading Stations™ Expanded to Uniaxial Strain 2

General Loading Station™ Information 3
Inserting Loading Stations™ into the BioFlex® Baseplate 3
Lubricant Application 3
Lubricant Removal 4

Equibiaxial Strain with Loading Stations™ and BioFlex® Culture Plates 4
Assigning and Downloading Regimens with the Equibiaxial Loading Stations™ 4
Equibiaxial Loading Station™ Specifications 4
Plating and Viewing Cells with the Equibiaxial Loading Stations™ 5

Equibiaxial Strain with 24-Well High Throughput Culture Plates 5
Assigning and Downloading Regimens with the 24-well Loading Stations™ 5
24-well Loading Station™ Specifications 5

Uniaxial Strain with Arctangle® Loading Stations™ and Either Tissue Train® or UniFlex® Culture Plates 6
Assigning and Downloading Regimens with the Arctangle® Loading Stations™ 6
Arctangle® Loading Station™ Specifications 6

Application Note: Viewing Cells on BioFlex® Culture Plates 7
Appendix 1: BioFlex® 25 mm Loading Station™ Conversion Chart 8
Appendix 2: BioFlex® 28 mm Loading Station™ Conversion Chart 10
Appendix 3: BioFlex® 31 mm Loading Station™ Conversion Chart 11
Appendix 4: Tissue Train® with Arctangle® Loading Station™ Conversion Chart 12
Appendix 5: UniFlex® with Arctangle® Loading Station™ Conversion Chart 13
Appendix 6: HT BioFlex® with 24-well Loading Station™ Conversion Chart 14
Appendix 7: BioFlex® with no Loading Station™ Conversion Chart 15

INTRODUCTION

PRINCIPLE OF LOADING STATIONS™ AND EQUIBIAXIAL STRAIN

BioFlex® Loading Stations™ provide uniform radial and circumferential strains to cells cultured on flexible membranes. Loading Stations™ are designed for use with BioFlex®, flexible-bottomed culture plates and the Flexcell® Tension system to provide regulated strain to cultured cells. The Loading Stations™ are comprised of a 3.3" x 5" Lexan® plate and six removable Delrin® planar faced cylindrical (28 mm, or 31 mm diameter; Fig. 1) or Arctangle® loading posts. The 25 mm planar faced cylindrical posts are made of VisiJet® material (a diacrylate compound). The posts are positioned on the Lexan® plate such that each is centered beneath the rubber membrane of each well of a 35 mm BioFlex® culture plate (Fig. 2). When vacuum is applied to a BioFlex® culture plate with a Flexcell® Tension System, the membrane deforms across the post face creating uniform equibiaxial strain (Fig. 2).

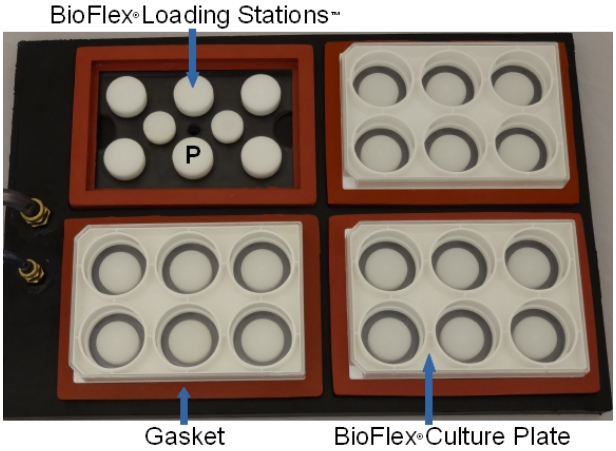

Figure 1. *BioFlex® baseplate showing the Loading Stations™ with six loading posts (P) and BioFlex® culture plates in red, rubber gaskets*

NOTE: *Flexcell® culture plates are good for 1 year when stored at room temperature in the dark or out of direct light.*

Loading posts are available in three standard diameters: 25, 28, and 31 mm. Use of loading posts provides: 1) constrained distension to the flexible membrane, and 2) nominal fluid shear stress because the medium is not moving up and down over the field. A silicone-based lubricant is used to minimize friction between the membrane and post.

NOTE: *Flexcell® ships the 25 mm Loading Stations™ as standard with the FX-5000™ Tension system unless the 28 mm or 31 mm Loading Stations™ are requested upon ordering.*

See Application Note in Appendix 1 for information regarding viewing cells on an inverted microscope.

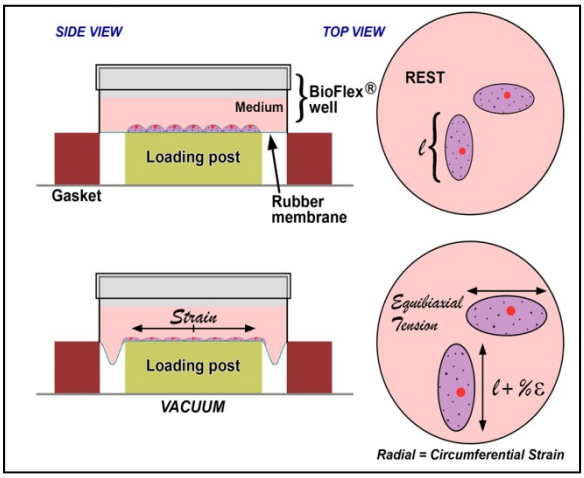

Figure 2. *Schematic of the BioFlex® membrane deformed across a loading post*

24-well High Throughput Plates. The 24-well HT BioFlex® plate (Fig.3) is designed with a flexible silicone membrane well bottom and with standard industry perimeter dimensions. The standard size allows the plate to be utilized with plate readers and

other standard culture plate devices. The total growth surface area for these plates is 37.47 cm^2 (or 1.56 cm^2/well). The 24-well Loading Stations™ have 10 mm diameter loading posts made of polystyrene. Equibiaxial strain is applied in the same manner as depicted in figure 2.

PRINCIPLE OF LOADING STATIONS™ EXPANDED TO UNIAXIAL STRAIN

3D Tissue Constructs with Tissue Train®. The above concept of strain application has been expanded to enable uniaxial strain to

Figure 3. *24-Well HT BioFlex® culture plate*

both 3-dimensional tissue cultures and monolayer cell cultures. Uniaxial strain is achieved through selectively controlling the portion of the flexible membrane that is exposed to the regulated vacuum. This is achieved through the use of special designed Arctangle® Loading Stations™ (Fig. 4). In addition to the Arctangle® Loading Stations™, specially configured culture plates are required to facilitate the uniaxial strain to the cell or tissue cultures. Tissue Train® culture plates are used in combination with Arctangle® Loading Stations™ (Fig. 5) to achieve uniaxial strain in a 3-dimensional tissue construct. Please note that the Trough Loader™ also shown in Figure 5 is used to develop three-dimensional tissue constructs.

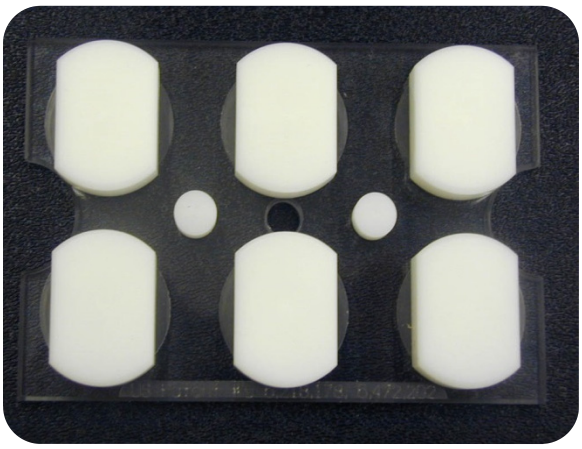

Figure 4. *Arctangle® Loading Stations™*

NOTE: *The development of three-dimensional tissue cultures is beyond the scope of this manual and is discussed in greater detail in Flexcell®'s "Tech Report 100: Tissue Train® Culture System".*

Figure 5. *Tissue Train® culture plate from 3D culture of cell-matrix constructs. The top left well has a Trough Loader™ beneath the flexible membrane. The 4 adjacent wells show the anchors for attachment of cells and gel from a uniaxial, linear construct. The bottom left well shows an Arctangle® loading post to deliver uniaxial load*

Monolayer Cell Cultures with UniFlex®. Uniaxial strain is achieved in monolayer cell cultures through the combined use of UniFlex® culture plates and Arctangle® Loading Stations™ (Fig. 6 & 7). UniFlex® culture plates are 35 mm 6-well culture plates

with the same overall dimensional configuration as the BioFlex® and Tissue Train® culture plates. Uniaxial strain is achieved on a centrally located rectangular portion of the UniFlex® well (Fig. 8). The dimensions of this region are 15.25 mm x 24.18 mm (0.600" x 0.952"). The uniaxial strain orientation is along the 24.18 mm axis. This region provides a total uniaxial strain area of 3.68 cm^2 (0.57 in^2).

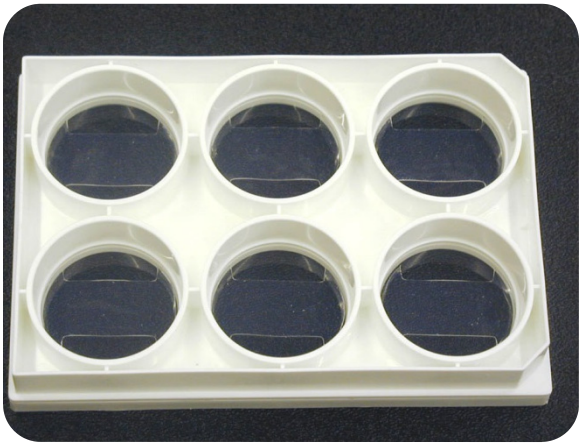

Figure 6. *UniFlex® flexible bottomed cell culture plate*

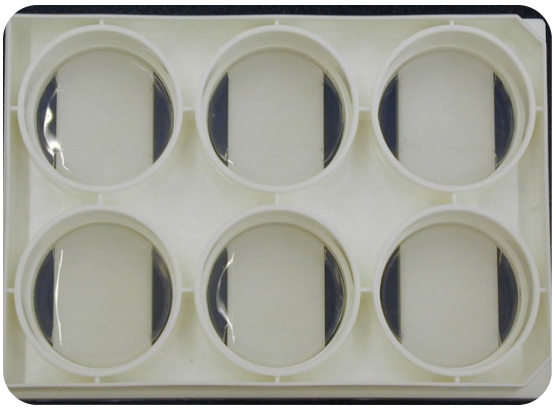

Figure 7. *UniFlex® Culture Plate on Arctangle® Loading Stations™*

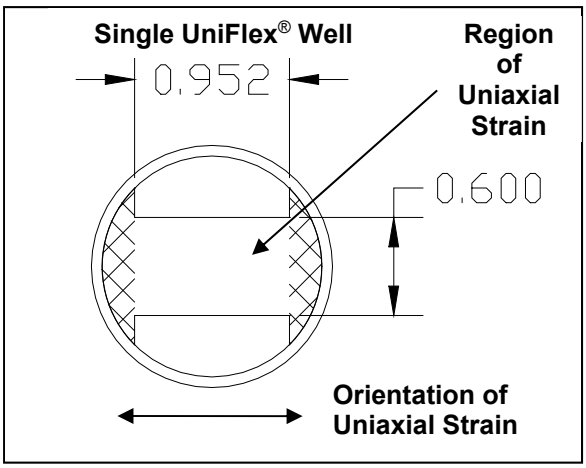

Figure 8. *Dimensioned UniFlex® well showing region and orientation of uniaxial strain. Total area = 3.68 cm².*

GENERAL LOADING STATION™ INFORMATION

INSERTING LOADING STATIONS™ INTO THE BIOFLEX® BASEPLATE

All styles except the 25mm Loading Station™ consists of one Lexan® plate, two Delrin® support and centering posts, and six Delrin® strain posts. The 25mm strain posts and support and centering posts are made from VisiJet® (a diacrylate compound). The two support and centering posts are intended to support the BioFlex® plate under high vacuum and also center the BioFlex® plate over the six strain posts. The six strain posts provide the strain surface. This strain surface is either equibiaxial in the case of the round loading posts or uniaxial in the case of the Arctangle® loading posts. Four complete Loading Stations™ are inserted into the four BioFlex® baseplate wells.

LUBRICANT APPLICATION

Once the Loading Stations™ are placed within the BioFlex® baseplate wells, lubricant should be applied to the tops and sides of the six strain posts on each Loading

Station™. Use the Loctite® silicone lubricant supplied with the Loading Stations™. Use your finger to evenly spread a generous layer of lubricant over the top and side of each strain post. Be sure that the film is not thin but also not over-applied. The idea is to create a grease boundary layer that will minimize friction between the BioFlex® membrane and post surface. If the lubricant amount is too little the friction will not be minimized. If the lubricant amount is too great, the lubricant will gather up under the membrane during stretching and create a lump. Once the BioFlex® plates have been plated and the cells are ready for stretching, place the plates and gaskets into the BioFlex® wells. Place the cover and weights over top of the plates.

NOTE: For each new experiment, be sure to clean and re-lubricate the Loading Stations™.

LUBRICANT REMOVAL

Upon completion of a strain regimen, the Loctite® silicone lubricant may be removed from the culture plate's flexible bottomed membrane to facilitate microscopy viewing. Use the following procedure to remove the lubricant (perform this procedure ***after*** cells are fixed):

1) Using a dry cotton swab, wipe off as much of the excess lubricant as possible. This may take a couple of swabs. Only a thin film of lubricant should remain on the membrane surface.

2) Wet the tip of a new cotton swab with a mild cleaning solution such as Formula 409®, window cleaner, or hand soap. Rub the wet swab gently over the entire surface of the well. Repeat with a fresh cleaning solution as necessary to remove all residual lubricant from the membrane surface.

3) Rinse the cleaned surface with deionized water using a spray, wash, or dispensing bottle. Blot the surface dry with a Kimwipe® or other lint-free wipe.

EQUIBIAXIAL STRAIN WITH LOADING STATIONS™ AND BIOFLEX® CULTURE PLATES

ASSIGNING AND DOWNLOADING REGIMENS WITH THE EQUIBIAXIAL LOADING STATIONS™

If 25 mm Loading Stations™ are desired, select the ***BioFlex Loading Station (25mm)*** option under ***Platforms*** during regimen assignment. For the 28 mm or 31 mm Loading Stations™, choose ***BioFlex Loading Station (28mm)*** or ***BioFlex Loading Station (31mm)***, respectively.

Important Note: *Failing to select the appropriate **Platform** assignment will produce inaccurate elongation values. The **Platform** assignment must match the actual culture plate and Loading Station™ configuration being used for the desired strain to be applied.*

EQUIBIAXIAL LOADING STATION™ SPECIFICATIONS

The equibiaxial Loading Stations™ have maximum and minimum strain capabilities with respect to vacuum level. The following values are minimum and maximum % elongations for each of the three equibiaxial Loading Station™ sizes:

25 mm Diameter Loading Stations™ (equibiaxial strain):
Minimum achievable % elongation with the FX-5000™: 0.8%

Maximum achievable % elongation with the FX-5000™: 23.0%

28 mm Diameter Loading Stations™ (equibiaxial strain):
Minimum achievable % elongation with the FX-5000™: 1.0%
Maximum achievable % elongation with the FX-5000™: 15.9%

31 mm Diameter Loading Stations™ (equibiaxial strain):
Minimum achievable % elongation with the FX-5000™: 0.8%
Maximum achievable % elongation with the FX-5000™: 6.0%

When programming regimens, do not exceed these values in the *min%* and *max%* boxes for the equibiaxial Loading Station™ setup that you are using.

PLATING AND VIEWING CELLS WITH THE EQUIBIAXIAL LOADING STATIONS™

It should be noted that the only cells that receive uniform strain are those attached to the area of the membrane over the post when the membrane is in its fully stretched position. Therefore, it is best to attempt to plate cells only in the uniformly strained area or to view or test the cells that are only in the uniformly strained area. To determine this area, the following equation can be used:

Diameter = (Diameter of Loading Station™) / (1 + (Max%Elongation/100)),

where *Max%Elongation* is the maximum % elongation that you plan to use in your regimen and *diameter* is the diameter of the circle at the center of the membrane. Any cells outside of this circle will not receive uniform strain.

EQUIBIAXIAL STRAIN WITH 24-WELL HIGH THROUGHPUT CULTURE PLATES

ASSIGNING AND DOWNLOADING REGIMENS WITH THE 24-WELL LOADING STATIONS™

FX-5000™ Tension Systems are preloaded with all 24-well Loading Station™ parameters. For 24-well HT BioFlex® plates, select the ***HT 24-Well Plate (Cylindrical LS)*** option under ***Platform*** during regimen assignment.

Important Note: *Failing to select the appropriate **Platform** assignment will produce inaccurate elongation values. The **Platform** assignment must match the actual culture plate and Loading Station™ configuration being used for the desired strain to be applied.*

24-WELL LOADING STATION™ SPECIFICATIONS

The 24-well Loading Stations™ have maximum and minimum strain capabilities with respect to vacuum level. The following values are minimum and maximum % elongations for the HT BioFlex® plates:

HT BioFlex® w/ 24-well Loading Stations™ (equibiaxial strain):
Minimum achievable % elongation with the FX-5000™: 1.2%
Maximum achievable % elongation with the FX-5000™: 8.0%

When programming regimens, do not exceed these values in the *min%* and *max%* boxes for the 24-well Loading Station™ setup that you are using.

WARNING: Flexcell recommends that customers do not use HT BioFlex® plates for high level strains greater than 8% (50 kPA). The reason for this limitation is that the elastic material properties of the 0.010 inch thick silicone elastomer membrane used in this culture plate application will be exceeded, leading to irreversible plastic deformation and membrane failure during cyclic flexing regimens. In addition, these plates cannot be used under strain conditions without the 24-well Loading Station™. Putting these plates under vacuum in the absence of a Loading Station™ in unconstrained distension can also result in tearing of the well membrane for the same reasons

UNIAXIAL STRAIN WITH ARCTANGLE® LOADING STATIONS™ AND EITHER TISSUE TRAIN® OR UNIFLEX® CULTURE PLATES

ASSIGNING AND DOWNLOADING REGIMENS WITH THE ARCTANGLE® LOADING STATIONS™

FX-5000™ Tension Systems are preloaded with all Arctangle® Loading Station™ parameters. If Tissue Train® plates are desired, select the ***Tissue Train Plate (24mm Arctangle LS)*** option under ***Platform*** during regimen assignment. For the UniFlex® plate, select ***UniFlex Plate (24mm Arctangle LS)***.

Important Note: *Failing to select the appropriate **Platform** assignment will produce inaccurate elongation values. The **Platform** assignment must match the actual culture plate and Loading Station™ configuration being used for the desired strain to be applied.*

ARCTANGLE® LOADING STATION™ SPECIFICATIONS

The Arctangle® Loading Stations™ have maximum and minimum strain capabilities with respect to vacuum level. The following values are minimum and maximum % elongations for the Tissue Train® and UniFlex® plates:

Tissue Train® w/ Arctangle® Loading Stations™ (uniaxial strain):
Minimum achievable % elongation with the FX-5000™: 1.6%
Maximum achievable % elongation with the FX-5000™: 20.8%

UniFlex® w /Arctangle® Loading Stations™ (uniaxial strain):
Minimum achievable % elongation with the FX-5000™: 1.1%
Maximum achievable % elongation with the FX-5000™: 12.2%

When programming regimens, do not exceed these values in the *min%* and *max%* boxes for the Arctangle® Loading Station™ setup that you are using.

APPLICATION NOTE: VIEWING CELLS ON BIOFLEX® CULTURE PLATES

An inverted phase microscope or other cell culture microscope allows viewing of the cells from the underside of the membrane. The cell-growth surface of the BioFlex® culture plate is slightly higher than other culture plates, and thus is more distant from the microscope objective. To view cells on the growth surface of the BioFlex® membrane, one can adjust the stop on the nosepiece carriage so that the objective lens can be focused on the membrane. Alternatively, a spacer ring can also be placed between the microscope objective nosepiece and the objective.

Therefore, viewing cells on the BioFlex® culture plate with an inverted microscope may require minor modifications to the microscope. Given this problem, here are two solutions:

1. *Adjust the vertical travel stop mechanism from the focus adjustment on your microscope*. Most microscopes have a screw mechanism to stop the vertical travel point relative to the stage (*a*, Fig. 9). A stop point prevents the objective from contacting the culture plate on the stage. If this stop mechanism is adjusted, the objective lens can travel further allowing the objective to focus on cells on the BioFlex® membrane. Different microscopes have different mechanisms for adjusting the distance for objective lens travel. If you are not sure of the location of your travel adjustment mechanism, contact your local microscope representative or the manufacturer for assistance.

2. *Use a spacer or expansion ring to extend the focal length of the objective lens*. A spacer or expansion ring is a screw-in ring located between the microscope nosepiece and objective lens. Addition of a spacer ring will increase the focal length of the objective lens. Contact your microscope manufacturer if you wish to use one of these. Using a spacer may decrease parfocality and centration in viewing cells.

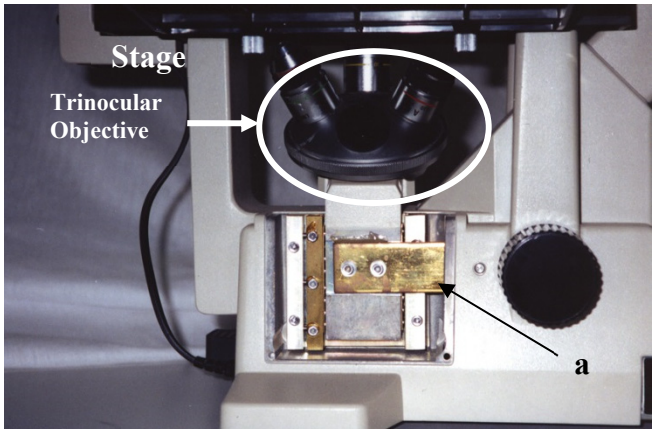

Figure 9. *Side view of an Olympus CK2 microscope; screw stop mechanism (a) will normally stop movement of the horizontal brass piece at its far right side*

Before adjusting your microscope, contact the manufacturer. Flexcell® International Corporation is not responsible for any damaged caused to your microscope.

APPENDIX 1: BIOFLEX® 25 MM LOADING STATION™ CONVERSION CHART

Press (-kPa)	% Elong	Strain	Press (-kPa)	% Elong	Strain	Press (-kPa)	% Elong	Strain
0.00	0.0	0.000	28.13	5.0	0.050	49.56	10.0	0.100
0.64	0.1	0.001	28.62	5.1	0.051	49.93	10.1	0.101
1.28	0.2	0.002	29.10	5.2	0.052	50.31	10.2	0.102
1.92	0.3	0.003	29.58	5.3	0.053	50.68	10.3	0.103
2.55	0.4	0.004	30.06	5.4	0.054	51.05	10.4	0.104
3.18	0.5	0.005	30.54	5.5	0.055	51.42	10.5	0.105
3.80	0.6	0.006	31.01	5.6	0.056	51.78	10.6	0.106
4.42	0.7	0.007	31.48	5.7	0.057	52.15	10.7	0.107
5.04	0.8	0.008	31.95	5.8	0.058	52.51	10.8	0.108
5.66	0.9	0.009	32.42	5.9	0.059	52.87	10.9	0.109
6.27	1.0	0.010	32.88	6.0	0.060	53.23	11.0	0.110
6.87	1.1	0.011	33.34	6.1	0.061	53.59	11.1	0.111
7.48	1.2	0.012	33.80	6.2	0.062	53.95	11.2	0.112
8.08	1.3	0.013	34.26	6.3	0.063	54.30	11.3	0.113
8.68	1.4	0.014	34.71	6.4	0.064	54.65	11.4	0.114
9.27	1.5	0.015	35.16	6.5	0.065	55.01	11.5	0.115
9.86	1.6	0.016	35.61	6.6	0.066	55.36	11.6	0.116
10.45	1.7	0.017	36.06	6.7	0.067	55.71	11.7	0.117
11.04	1.8	0.018	36.50	6.8	0.068	56.05	11.8	0.118
11.62	1.9	0.019	36.94	6.9	0.069	56.40	11.9	0.119
12.20	2.0	0.020	37.38	7.0	0.070	56.74	12.0	0.120
12.77	2.1	0.021	37.82	7.1	0.071	57.08	12.1	0.121
13.34	2.2	0.022	38.26	7.2	0.072	57.43	12.2	0.122
13.91	2.3	0.023	38.69	7.3	0.073	57.77	12.3	0.123
14.48	2.4	0.024	39.12	7.4	0.074	58.10	12.4	0.124
15.04	2.5	0.025	39.55	7.5	0.075	58.44	12.5	0.125
15.60	2.6	0.026	39.97	7.6	0.076	58.78	12.6	0.126
16.15	2.7	0.027	40.39	7.7	0.077	59.11	12.7	0.127
16.71	2.8	0.028	40.82	7.8	0.078	59.44	12.8	0.128
17.26	2.9	0.029	41.24	7.9	0.079	59.78	12.9	0.129
17.80	3.0	0.030	41.65	8.0	0.080	60.11	13.0	0.130
18.35	3.1	0.031	42.07	8.1	0.081	60.43	13.1	0.131
18.89	3.2	0.032	42.48	8.2	0.082	60.76	13.2	0.132
19.43	3.3	0.033	42.89	8.3	0.083	61.09	13.3	0.133
19.96	3.4	0.034	43.30	8.4	0.084	61.41	13.4	0.134
20.49	3.5	0.035	43.70	8.5	0.085	61.74	13.5	0.135
21.02	3.6	0.036	44.11	8.6	0.086	62.06	13.6	0.136
21.55	3.7	0.037	44.51	8.7	0.087	62.38	13.7	0.137
22.07	3.8	0.038	44.91	8.8	0.088	62.70	13.8	0.138
22.59	3.9	0.039	45.31	8.9	0.089	63.02	13.9	0.139
23.11	4.0	0.040	45.70	9.0	0.090	63.34	14.0	0.140
23.62	4.1	0.041	46.10	9.1	0.091	63.66	14.1	0.141
24.14	4.2	0.042	46.49	9.2	0.092	63.97	14.2	0.142
24.64	4.3	0.043	46.88	9.3	0.093	64.29	14.3	0.143
25.15	4.4	0.044	47.27	9.4	0.094	64.60	14.4	0.144
25.65	4.5	0.045	47.66	9.5	0.095	64.91	14.5	0.145
26.15	4.6	0.046	48.04	9.6	0.096	65.23	14.6	0.146
26.65	4.7	0.047	48.42	9.7	0.097	65.54	14.7	0.147
27.15	4.8	0.048	48.80	9.8	0.098	65.85	14.8	0.148
27.64	4.9	0.049	49.18	9.9	0.099	66.16	14.9	0.149

BIOFLEX® 25 MM LOADING STATION™ CONVERSION CHART

Press (-kPa)	% Elong	Strain	Press (-kPa)	% Elong	Strain
66.46	15.0	0.150	81.02	20.0	0.200
66.77	15.1	0.151	81.30	20.1	0.201
67.08	15.2	0.152	81.58	20.2	0.202
67.38	15.3	0.153	81.86	20.3	0.203
67.69	15.4	0.154	82.15	20.4	0.204
67.99	15.5	0.155	82.43	20.5	0.205
68.29	15.6	0.156	82.71	20.6	0.206
68.59	15.7	0.157	82.99	20.7	0.207
68.89	15.8	0.158	83.27	20.8	0.208
69.19	15.9	0.159	83.56	20.9	0.209
69.49	16.0	0.160	83.84	21.0	0.210
69.79	16.1	0.161	84.12	21.1	0.211
70.09	16.2	0.162	84.40	21.2	0.212
70.39	16.3	0.163	84.69	21.3	0.213
70.68	16.4	0.164	84.97	21.4	0.214
70.98	16.5	0.165	85.25	21.5	0.215
71.27	16.6	0.166	85.53	21.6	0.216
71.57	16.7	0.167	85.82	21.7	0.217
71.86	16.8	0.168	86.10	21.8	0.218
72.15	16.9	0.169	86.38	21.9	0.219
72.45	17.0	0.170	86.67	22.0	0.220
72.74	17.1	0.171	86.95	22.1	0.221
73.03	17.2	0.172	87.24	22.2	0.222
73.32	17.3	0.173	87.52	22.3	0.223
73.61	17.4	0.174	87.81	22.4	0.224
73.90	17.5	0.175	88.09	22.5	0.225
74.19	17.6	0.176	88.38	22.6	0.226
74.48	17.7	0.177	88.67	22.7	0.227
74.76	17.8	0.178	88.95	22.8	0.228
75.05	17.9	0.179	89.24	22.9	0.229
75.34	18.0	0.180	89.53	23.0	0.230
75.63	18.1	0.181			
75.91	18.2	0.182			
76.20	18.3	0.183			
76.48	18.4	0.184			
76.77	18.5	0.185			
77.05	18.6	0.186			
77.34	18.7	0.187			
77.62	18.8	0.188			
77.91	18.9	0.189			
78.19	19.0	0.190			
78.47	19.1	0.191			
78.76	19.2	0.192			
79.04	19.3	0.193			
79.32	19.4	0.194			
79.61	19.5	0.195			
79.89	19.6	0.196			
80.17	19.7	0.197			
80.45	19.8	0.198			
80.74	19.9	0.199			

APPENDIX 2: BIOFLEX® 28 MM LOADING STATION™ CONVERSION CHART

Press (-kPa)	% Elong	Strain	Press (-kPa)	% Elong	Strain	Press (-kPa)	% Elong	Strain
0.00	0.0	0.000	36.29	5.4	0.054	63.73	10.8	0.108
0.81	0.1	0.001	36.85	5.5	0.055	64.21	10.9	0.109
1.61	0.2	0.002	37.41	5.6	0.056	64.70	11.0	0.110
2.40	0.3	0.003	37.97	5.7	0.057	65.18	11.1	0.111
3.19	0.4	0.004	38.52	5.8	0.058	65.66	11.2	0.112
3.97	0.5	0.005	39.07	5.9	0.059	66.14	11.3	0.113
4.75	0.6	0.006	39.61	6.0	0.060	66.63	11.4	0.114
5.52	0.7	0.007	40.16	6.1	0.061	67.11	11.5	0.115
6.29	0.8	0.008	40.70	6.2	0.062	67.60	11.6	0.116
7.05	0.9	0.009	41.24	6.3	0.063	68.08	11.7	0.117
7.80	1.0	0.010	41.77	6.4	0.064	68.57	11.8	0.118
8.55	1.1	0.011	42.31	6.5	0.065	69.05	11.9	0.119
9.30	1.2	0.012	42.84	6.6	0.066	69.54	12.0	0.120
10.03	1.3	0.013	43.37	6.7	0.067	70.03	12.1	0.121
10.77	1.4	0.014	43.89	6.8	0.068	70.51	12.2	0.122
11.49	1.5	0.015	44.42	6.9	0.069	71.00	12.3	0.123
12.21	1.6	0.016	44.94	7.0	0.070	71.49	12.4	0.124
12.93	1.7	0.017	45.46	7.1	0.071	71.99	12.5	0.125
13.64	1.8	0.018	45.98	7.2	0.072	72.48	12.6	0.126
14.35	1.9	0.019	46.49	7.3	0.073	72.97	12.7	0.127
15.05	2.0	0.020	47.01	7.4	0.074	73.47	12.8	0.128
15.75	2.1	0.021	47.52	7.5	0.075	73.96	12.9	0.129
16.44	2.2	0.022	48.03	7.6	0.076	74.46	13.0	0.130
17.12	2.3	0.023	48.54	7.7	0.077	74.96	13.1	0.131
17.80	2.4	0.024	49.04	7.8	0.078	75.46	13.2	0.132
18.48	2.5	0.025	49.55	7.9	0.079	75.96	13.3	0.133
19.15	2.6	0.026	50.05	8.0	0.080	76.46	13.4	0.134
19.82	2.7	0.027	50.55	8.1	0.081	76.96	13.5	0.135
20.48	2.8	0.028	51.05	8.2	0.082	77.47	13.6	0.136
21.14	2.9	0.029	51.55	8.3	0.083	77.98	13.7	0.137
21.80	3.0	0.030	52.05	8.4	0.084	78.49	13.8	0.138
22.45	3.1	0.031	52.55	8.5	0.085	79.00	13.9	0.139
23.09	3.2	0.032	53.04	8.6	0.086	79.51	14.0	0.140
23.73	3.3	0.033	53.53	8.7	0.087	80.03	14.1	0.141
24.37	3.4	0.034	54.03	8.8	0.088	80.54	14.2	0.142
25.00	3.5	0.035	54.52	8.9	0.089	81.06	14.3	0.143
25.63	3.6	0.036	55.01	9.0	0.090	81.58	14.4	0.144
26.25	3.7	0.037	55.50	9.1	0.091	82.11	14.5	0.145
26.87	3.8	0.038	55.99	9.2	0.092	82.63	14.6	0.146
27.49	3.9	0.039	56.48	9.3	0.093	83.16	14.7	0.147
28.10	4.0	0.040	56.96	9.4	0.094	83.69	14.8	0.148
28.71	4.1	0.041	57.45	9.5	0.095	84.22	14.9	0.149
29.31	4.2	0.042	57.93	9.6	0.096	84.76	15.0	0.150
29.91	4.3	0.043	58.42	9.7	0.097	85.30	15.1	0.151
30.51	4.4	0.044	58.90	9.8	0.098	85.84	15.2	0.152
31.10	4.5	0.045	59.39	9.9	0.099	86.38	15.3	0.153
31.69	4.6	0.046	59.87	10.0	0.100	86.92	15.4	0.154
32.28	4.7	0.047	60.36	10.1	0.101	87.47	15.5	0.155
32.86	4.8	0.048	60.84	10.2	0.102	88.02	15.6	0.156
33.44	4.9	0.049	61.32	10.3	0.103	88.57	15.7	0.157
34.02	5.0	0.050	61.80	10.4	0.104	89.13	15.8	0.158
34.59	5.1	0.051	62.29	10.5	0.105	89.69	15.9	0.159
35.16	5.2	0.052	62.77	10.6	0.106			
35.73	5.3	0.053	63.25	10.7	0.107			

APPENDIX 3: BIOFLEX® 31 MM LOADING STATION™ CONVERSION CHART

Press (-kPa)	% Elong	Strain	Press (-kPa)	% Elong	Strain
0.00	0.0	0.000	80.20	5.0	0.050
2.75	0.1	0.001	81.25	5.1	0.051
5.44	0.2	0.002	82.31	5.2	0.052
8.05	0.3	0.003	83.37	5.3	0.053
10.59	0.4	0.004	84.45	5.4	0.054
13.07	0.5	0.005	85.53	5.5	0.055
15.48	0.6	0.006	86.62	5.6	0.056
17.83	0.7	0.007	87.73	5.7	0.057
20.12	0.8	0.008	88.85	5.8	0.058
22.34	0.9	0.009	89.98	5.9	0.059
24.51	1.0	0.010			
26.62	1.1	0.011			
28.67	1.2	0.012			
30.67	1.3	0.013			
32.62	1.4	0.014			
34.51	1.5	0.015			
36.36	1.6	0.016			
38.15	1.7	0.017			
39.90	1.8	0.018			
41.61	1.9	0.019			
43.27	2.0	0.020			
44.88	2.1	0.021			
46.46	2.2	0.022			
48.00	2.3	0.023			
49.50	2.4	0.024			
50.96	2.5	0.025			
52.39	2.6	0.026			
53.78	2.7	0.027			
55.15	2.8	0.028			
56.48	2.9	0.029			
57.78	3.0	0.030			
59.06	3.1	0.031			
60.31	3.2	0.032			
61.54	3.3	0.033			
62.75	3.4	0.034			
63.93	3.5	0.035			
65.09	3.6	0.036			
66.24	3.7	0.037			
67.37	3.8	0.038			
68.49	3.9	0.039			
69.59	4.0	0.040			
70.68	4.1	0.041			
71.76	4.2	0.042			
72.83	4.3	0.043			
73.89	4.4	0.044			
74.95	4.5	0.045			
76.00	4.6	0.046			
77.05	4.7	0.047			
78.10	4.8	0.048			
79.15	4.9	0.049			

APPENDIX 4: TISSUE TRAIN® WITH ARCTANGLE® LOADING STATION™ CONVERSION CHART

Press (-kPa)	% Elong	Strain	Press (-kPa)	% Elong	Strain
0.00	0.0	0.000	52.62	12.0	0.120
1.47	0.2	0.002	53.24	12.2	0.122
2.91	0.4	0.004	53.85	12.4	0.124
4.32	0.6	0.006	54.47	12.6	0.126
5.69	0.8	0.008	55.09	12.8	0.128
7.04	1.0	0.010	55.72	13.0	0.130
8.36	1.2	0.012	56.35	13.2	0.132
9.65	1.4	0.014	56.99	13.4	0.134
10.92	1.6	0.016	57.64	13.6	0.136
12.16	1.8	0.018	58.29	13.8	0.138
13.37	2.0	0.020	58.95	14.0	0.140
14.55	2.2	0.022	59.62	14.2	0.142
15.71	2.4	0.024	60.30	14.4	0.144
16.84	2.6	0.026	60.99	14.6	0.146
17.95	2.8	0.028	61.68	14.8	0.148
19.04	3.0	0.030	62.39	15.0	0.150
20.10	3.2	0.032	63.11	15.2	0.152
21.14	3.4	0.034	63.84	15.4	0.154
22.16	3.6	0.036	64.58	15.6	0.156
23.16	3.8	0.038	65.34	15.8	0.158
24.13	4.0	0.040	66.11	16.0	0.160
25.09	4.2	0.042	66.89	16.2	0.162
26.02	4.4	0.044	67.69	16.4	0.164
26.94	4.6	0.046	68.50	16.6	0.166
27.84	4.8	0.048	69.33	16.8	0.168
28.72	5.0	0.050	70.17	17.0	0.170
29.58	5.2	0.052	71.03	17.2	0.172
30.43	5.4	0.054	71.91	17.4	0.174
31.26	5.6	0.056	72.81	17.6	0.176
32.07	5.8	0.058	73.72	17.8	0.178
32.87	6.0	0.060	74.66	18.0	0.180
33.66	6.2	0.062	75.61	18.2	0.182
34.43	6.4	0.064	76.58	18.4	0.184
35.18	6.6	0.066	77.58	18.6	0.186
35.93	6.8	0.068	78.59	18.8	0.188
36.66	7.0	0.070	79.63	19.0	0.190
37.38	7.2	0.072	80.69	19.2	0.192
38.09	7.4	0.074	81.77	19.4	0.194
38.79	7.6	0.076	82.88	19.6	0.196
39.47	7.8	0.078	84.01	19.8	0.198
40.15	8.0	0.080	85.17	20.0	0.200
40.82	8.2	0.082	86.35	20.2	0.202
41.48	8.4	0.084	87.56	20.4	0.204
42.14	8.6	0.086	88.79	20.6	0.206
42.78	8.8	0.088	90.05	20.8	0.208
43.42	9.0	0.090			
44.06	9.2	0.092			
44.69	9.4	0.094			
45.31	9.6	0.096			
45.93	9.8	0.098			
46.55	10.0	0.100			
47.16	10.2	0.102			
47.77	10.4	0.104			
48.38	10.6	0.106			
48.98	10.8	0.108			
49.59	11.0	0.110			
50.19	11.2	0.112			
50.80	11.4	0.114			
51.41	11.6	0.116			
52.01	11.8	0.118			

APPENDIX 5: UNIFLEX® WITH ARCTANGLE® LOADING STATION™ CONVERSION CHART

Press (-kPa)	% Elong	Strain	Press (-kPa)	% Elong	Strain
0.00	0.0	0.000	75.17	10.0	0.100
2.73	0.2	0.002	76.32	10.2	0.102
5.37	0.4	0.004	77.51	10.4	0.104
7.93	0.6	0.006	78.72	10.6	0.106
10.41	0.8	0.008	79.95	10.8	0.054
12.81	1.0	0.010	81.22	11.0	0.055
15.14	1.2	0.012	82.52	11.2	0.056
17.39	1.4	0.014	83.86	11.4	0.057
19.56	1.6	0.016	85.24	11.6	0.058
21.67	1.8	0.018	86.65	11.8	0.059
23.72	2.0	0.020	88.11	12.0	0.060
25.69	2.2	0.022	89.61	12.2	0.061
27.61	2.4	0.024			
29.46	2.6	0.026			
31.25	2.8	0.028			
32.99	3.0	0.030			
34.67	3.2	0.032			
36.30	3.4	0.034			
37.88	3.6	0.036			
39.42	3.8	0.038			
40.90	4.0	0.040			
42.35	4.2	0.042			
43.75	4.4	0.044			
45.11	4.6	0.046			
46.44	4.8	0.048			
47.73	5.0	0.050			
48.99	5.2	0.052			
50.22	5.4	0.054			
51.42	5.6	0.056			
52.60	5.8	0.058			
53.75	6.0	0.060			
54.88	6.2	0.062			
55.99	6.4	0.064			
57.08	6.6	0.066			
58.16	6.8	0.068			
59.23	7.0	0.070			
60.28	7.2	0.072			
61.33	7.4	0.074			
62.37	7.6	0.076			
63.41	7.8	0.078			
64.44	8.0	0.080			
65.48	8.2	0.082			
66.51	8.4	0.084			
67.56	8.6	0.086			
68.60	8.8	0.088			
69.66	9.0	0.090			
70.73	9.2	0.092			
71.81	9.4	0.094			
72.91	9.6	0.096			
74.03	9.8	0.098			

APPENDIX 6: HT BIOFLEX® WITH 24-WELL LOADING STATION™ CONVERSION CHART

Press (-kPa)	% Elong	Strain	Press (-kPa)	% Elong	Strain	Press (-kPa)	% Elong	Strain
0.00	0.0	0.000	36.43	5.1	0.051	60.58	10.2	0.102
0.96	0.1	0.001	36.95	5.2	0.052	61.06	10.3	0.103
1.91	0.2	0.002	37.48	5.3	0.053	61.55	10.4	0.104
2.84	0.3	0.003	37.99	5.4	0.054	62.04	10.5	0.105
3.77	0.4	0.004	38.50	5.5	0.055	62.54	10.6	0.106
4.68	0.5	0.005	39.01	5.6	0.056	63.04	10.7	0.107
5.58	0.6	0.006	39.52	5.7	0.057	63.54	10.8	0.108
6.47	0.7	0.007	40.02	5.8	0.058	64.05	10.9	0.109
7.34	0.8	0.008	40.51	5.9	0.059	64.56	11.0	0.110
8.21	0.9	0.009	41.01	6.0	0.060	65.08	11.1	0.111
9.06	1.0	0.010	41.50	6.1	0.061	65.60	11.2	0.112
9.91	1.1	0.011	41.99	6.2	0.062	66.12	11.3	0.113
10.74	1.2	0.012	42.47	6.3	0.063	66.65	11.4	0.114
11.56	1.3	0.013	42.95	6.4	0.064	67.19	11.5	0.115
12.38	1.4	0.014	43.43	6.5	0.065	67.73	11.6	0.116
13.18	1.5	0.015	43.90	6.6	0.066	68.27	11.7	0.117
13.97	1.6	0.016	44.38	6.7	0.067	68.82	11.8	0.118
14.75	1.7	0.017	44.85	6.8	0.068	69.38	11.9	0.119
15.52	1.8	0.018	45.32	6.9	0.069	69.94	12.0	0.120
16.28	1.9	0.019	45.78	7.0	0.070	70.50	12.1	0.121
17.04	2.0	0.020	46.25	7.1	0.071	71.08	12.2	0.122
17.78	2.1	0.021	46.71	7.2	0.072	71.65	12.3	0.123
18.51	2.2	0.022	47.17	7.3	0.073	72.24	12.4	0.124
19.24	2.3	0.023	47.63	7.4	0.074	72.83	12.5	0.125
19.95	2.4	0.024	48.09	7.5	0.075	73.43	12.6	0.126
20.66	2.5	0.025	48.55	7.6	0.076	74.03	12.7	0.127
21.36	2.6	0.026	49.01	7.7	0.077	74.64	12.8	0.128
22.04	2.7	0.027	49.46	7.8	0.078	75.26	12.9	0.129
22.73	2.8	0.028	49.92	7.9	0.079	75.89	13.0	0.130
23.40	2.9	0.029	50.37	8.0	0.080	76.52	13.1	0.131
24.06	3.0	0.030	50.83	8.1	0.081	77.16	13.2	0.132
24.72	3.1	0.031	51.28	8.2	0.082	77.81	13.3	0.133
25.37	3.2	0.032	51.74	8.3	0.083	78.46	13.4	0.134
26.01	3.3	0.033	52.19	8.4	0.084	79.12	13.5	0.135
26.64	3.4	0.034	52.65	8.5	0.085	79.80	13.6	0.136
27.27	3.5	0.035	53.10	8.6	0.086	80.47	13.7	0.137
27.89	3.6	0.036	53.56	8.7	0.087	81.16	13.8	0.138
28.50	3.7	0.037	54.02	8.8	0.088	81.86	13.9	0.139
29.11	3.8	0.038	54.47	8.9	0.089	82.56	14.0	0.140
29.71	3.9	0.039	54.93	9.0	0.090	83.28	14.1	0.141
30.30	4.0	0.040	55.39	9.1	0.091	84.00	14.2	0.142
30.89	4.1	0.041	55.86	9.2	0.092	84.73	14.3	0.143
31.47	4.2	0.042	56.32	9.3	0.093	85.47	14.4	0.144
32.04	4.3	0.043	56.78	9.4	0.094	86.22	14.5	0.145
32.61	4.4	0.044	57.25	9.5	0.095	86.98	14.6	0.146
33.17	4.5	0.045	57.72	9.6	0.096	87.75	14.7	0.147
33.73	4.6	0.046	58.19	9.7	0.097	88.53	14.8	0.148
34.28	4.7	0.047	58.66	9.8	0.098	89.32	14.9	0.149
34.82	4.8	0.048	59.14	9.9	0.099	90.12	15.0	0.150
35.36	4.9	0.049	59.61	10.0	0.100			
35.90	5.0	0.050	60.09	10.1	0.101			

APPENDIX 7: BIOFLEX® WITH NO LOADING STATION™ CONVERSION CHART

Press (-kPa)	% Elong	Strain	Press (-kPa)	% Elong	Strain	Press (-kPa)	% Elong	Strain
0.00	0.0	0.000	7.11	11.4	0.057	18.45	22.8	0.114
0.07	0.2	0.002	7.28	11.6	0.058	18.67	23.0	0.115
0.14	0.4	0.004	7.45	11.8	0.059	18.89	23.2	0.116
0.22	0.6	0.006	7.63	12.0	0.060	19.11	23.4	0.117
0.29	0.8	0.008	7.80	12.2	0.061	19.33	23.6	0.118
0.37	1.0	0.010	7.98	12.4	0.062	19.55	23.8	0.119
0.46	1.2	0.012	8.16	12.6	0.063	19.77	24.0	0.120
0.54	1.4	0.014	8.34	12.8	0.064	19.99	24.2	0.121
0.63	1.6	0.016	8.52	13.0	0.065	20.21	24.4	0.122
0.71	1.8	0.018	8.70	13.2	0.066	20.43	24.6	0.123
0.80	2.0	0.020	8.88	13.4	0.067	20.65	24.8	0.124
0.90	2.2	0.022	9.06	13.6	0.068	20.87	25.0	0.125
0.99	2.4	0.024	9.25	13.8	0.069	21.09	25.2	0.126
1.09	2.6	0.026	9.44	14.0	0.070	21.31	25.4	0.127
1.18	2.8	0.028	9.62	14.2	0.071	21.53	25.6	0.128
1.28	3.0	0.030	9.81	14.4	0.072	21.75	25.8	0.129
1.39	3.2	0.032	10.00	14.6	0.073	21.97	26.0	0.130
1.49	3.4	0.034	10.19	14.8	0.074	22.20	26.2	0.131
1.60	3.6	0.036	10.38	15.0	0.075	22.42	26.4	0.132
1.71	3.8	0.038	10.57	15.2	0.076	22.64	26.6	0.133
1.82	4.0	0.040	10.77	15.4	0.077	22.86	26.8	0.134
1.93	4.2	0.042	10.96	15.6	0.078	23.08	27.0	0.135
2.04	4.4	0.044	11.16	15.8	0.079	23.30	27.2	0.136
2.16	4.6	0.046	11.35	16.0	0.080	23.52	27.4	0.137
2.28	4.8	0.048	11.55	16.2	0.081	23.75	27.6	0.138
2.40	5.0	0.050	11.75	16.4	0.082	23.97	27.8	0.139
2.52	5.2	0.052	11.95	16.6	0.083	24.19	28.0	0.140
2.64	5.4	0.054	12.15	16.8	0.084	24.41	28.2	0.141
2.77	5.6	0.056	12.35	17.0	0.085	24.63	28.4	0.142
2.89	5.8	0.058	12.55	17.2	0.086	24.85	28.6	0.143
3.02	6.0	0.060	12.75	17.4	0.087	25.07	28.8	0.144
3.15	6.2	0.062	12.95	17.6	0.088	25.29	29.0	0.145
3.28	6.4	0.064	13.16	17.8	0.089	25.51	29.2	0.146
3.42	6.6	0.066	13.36	18.0	0.090	25.73	29.4	0.147
3.55	6.8	0.068	13.57	18.2	0.091	25.95	29.6	0.148
3.69	7.0	0.070	13.77	18.4	0.092	26.17	29.8	0.149
3.83	7.2	0.072	13.98	18.6	0.093	26.39	30.0	0.150
3.97	7.4	0.074	14.19	18.8	0.094	26.61	30.2	0.151
4.11	7.6	0.076	14.39	19.0	0.095	26.83	30.4	0.152
4.26	7.8	0.078	14.60	19.2	0.096	27.05	30.6	0.153
4.40	8.0	0.080	14.81	19.4	0.097	27.27	30.8	0.154
4.55	8.2	0.082	15.02	19.6	0.098	27.49	31.0	0.155
4.70	8.4	0.084	15.23	19.8	0.099	27.70	31.2	0.156
4.85	8.6	0.086	15.44	20.0	0.100	27.92	31.4	0.157
5.00	8.8	0.088	15.66	20.2	0.101	28.14	31.6	0.158
5.15	9.0	0.090	15.87	20.4	0.102	28.36	31.8	0.159
5.31	9.2	0.092	16.08	20.6	0.103	28.57	32.0	0.160
5.47	9.4	0.094	16.29	20.8	0.104	28.79	32.2	0.161
5.62	9.6	0.096	16.51	21.0	0.105	29.00	32.4	0.134
5.78	9.8	0.098	16.72	21.2	0.106	29.22	32.6	0.135
5.94	10.0	0.100	16.94	21.4	0.107	29.43	32.8	0.136
6.11	10.2	0.102	17.15	21.6	0.108	29.65	33.0	0.137
6.27	10.4	0.104	17.37	21.8	0.109	29.86	33.2	0.138
6.43	10.6	0.106	17.59	22.0	0.110	30.07	33.4	0.139
6.60	10.8	0.054	17.80	22.2	0.111			
6.77	11.0	0.055	18.02	22.4	0.112			
6.94	11.2	0.056	18.24	22.6	0.113			