Table 1, Suggested Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	5 µg	5 µg	HAase Control	HAase Control	Sample 7	Sample 7	Sample 15	Sample 15	Sample 23	Sample 23	Sample 31	Sample 31
B	2.5 µg	2.5 µg	No HAase	No HAase	Sample 8	Sample 8	Sample 16	Sample 16	Sample 24	Sample 24	Sample 32	Sample 32
C	1.25 µg	1.25 µg	Sample 1	Sample 1	Sample 9	Sample 9	Sample 17	Sample 17	Sample 25	Sample 25	Sample 33	Sample 33
D	0.625 µg	0.625 µg	Sample 2	Sample 2	Sample 10	Sample 10	Sample 18	Sample 18	Sample 26	Sample 26	Sample 34	Sample 34
E	0.313 µg	0.313 µg	Sample 3	Sample 3	Sample 11	Sample 11	Sample 19	Sample 19	Sample 27	Sample 27	Sample 35	Sample 35
F	0.156 µg	0.156 µg	Sample 4	Sample 4	Sample 12	Sample 12	Sample 20	Sample 20	Sample 28	Sample 28	Sample 36	Sample 36
G	0.078 µg	0.078 µg	Sample 5	Sample 5	Sample 13	Sample 13	Sample 21	Sample 21	Sample 29	Sample 29	Sample 37	Sample 37
H	No HA	No HA	Sample 6	Sample 6	Sample 14	Sample 14	Sample 22	Sample 22	Sample 30	Sample 30	Sample 38	Sample 38

Data Analysis

Generate a best fit curve for the HA substrate standards and interpolate the remaining HA substrate in the reaction wells. Hyaluronidase activity can then be determined based on amount of HA substrate removed during the reaction time period.

Graph the standard curve (column 1 and 2 of the plate) as OD vs ug HA remaining. Then fit a nonlinear 4-parameter curve. The sample OD values are then interpolated against the standard curve. Once the amount of HA remaining for each samples is determined it can be plugged into the equation below.

% HA removed = (1- (ug HA remaining in sample/5ug)) X 100

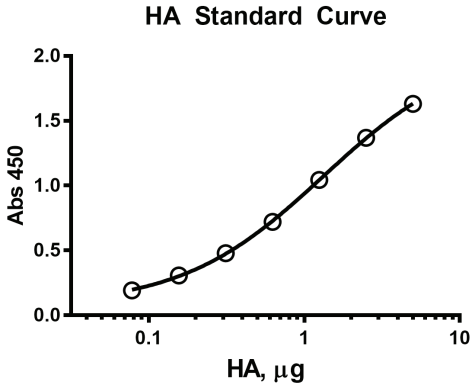

Figure 1. HA standard curve was generated using non-linear regression analysis with GraphPad Software. A log[agonist] vs. response-variable slope (four parameter) analysis was utilized.

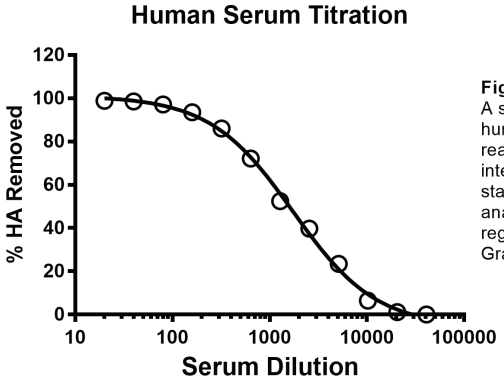

Figure 2. A sample titration using human serum with a 2 hour reaction time. Data interpolated against an HA standard curve and analyzed using non-linear regression analysis with GraphPad Software.

References

1. Chang S-H, Yeh Y-H, Lee J-L, Hsu Y-J, Kuo C-T, Chen W-J. Transforming growth factor-β-mediated CD44/STAT3 signaling contributes to the development of atrial fibrosis and fibrillation. Basic Research in Cardiology. 2017;112(5):58. doi: 10.1007/s00395-017-0647-9.
2. Margraf A, Herter JM, Kühne K, Stadtmann A, Ermert T, Wenk M, et al. 6% Hydroxyethyl starch (HES 130/0.4) diminishes glycocalyx degradation and decreases vascular permeability during systemic and pulmonary inflammation in mice. Critical Care. 2018;22(1):111.

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