Application Note:
Processing Absorbance Data Obtained Using PrestoBlue™ Viability Reagent

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This technical note provides guidance for analyzing absorbance data obtained using PrestoBlue™ Viability Reagent on an absorbance-based plate reader or spectrophotometer. Given the nature in which certain absorbance-based plate readers or spectrophotometers normalize data, negative values may be obtained for samples in which there is low conversion of PrestoBlue™ Viability Reagent. This is expected, and the data obtained is still valid.

Data Calculation

1) After proper incubation of your assay plate, read the absorbance of all wells at both the 570 nm and 600 nm wavelengths.
    Note: Many absorbance plate readers will ask for a reference or normalization wavelength. In this case, use 570 nm as the experimental wavelength and 600 nm as the reference or normalization wavelength.

Raw Data: 570 nm

Cells/Well	100,000	50,000	25,000	12,500	6,250	3,125	1,563	781	391	195	98	Media Only
<>	1	2	3	4	5	6	7	8	9	10	11	12
A	1.2912	1.1252	1.0319	0.9776	0.9456	0.9352	0.9130	0.9238	0.9221	0.9256	0.9367	0.9550
B	1.3043	1.1300	1.0306	0.9764	0.9376	0.9236	0.9169	0.9330	0.9213	0.9301	0.9255	0.9660
C	1.2815	1.1308	1.0388	0.9807	0.9511	0.9374	0.9279	0.9164	0.9094	0.9289	0.9466	0.9516
D	1.3075	1.1242	1.0503	0.9825	0.9395	0.9269	0.9092	0.9013	0.9072	0.9034	0.9234	0.9681
E	1.2890	1.1142	1.0450	0.9753	0.9493	0.9102	0.9208	0.9147	0.9065	0.9454	0.9588	0.9702
F	1.2902	1.1225	1.0373	0.9697	0.9447	0.9158	0.8938	0.9146	0.8068	0.9190	0.9297	0.9769
G	1.2975	1.1308	1.0322	0.9919	0.9555	0.9138	0.6877	0.9207	0.8261	0.7768	0.9227	0.9633
H	1.3029	1.1154	1.0303	0.9685	0.9318	0.9016	0.7950	0.8940	0.9097	0.7687	0.7752	0.9097

Raw Data: 600 nm

Cells/Well	100,000	50,000	25,000	12,500	6,250	3,125	1,563	781	391	195	98	Media Only
<>	1	2	3	4	5	6	7	8	9	10	11	12
A	0.6948	0.8063	0.9234	1.0172	1.0584	1.0856	1.0877	1.1061	1.1099	1.1186	1.1367	1.1508
B	0.7241	0.7961	0.9297	1.0116	1.0600	1.0871	1.1043	1.1294	1.1215	1.1328	1.1312	1.1676
C	0.6979	0.7954	0.9275	1.0191	1.0707	1.1038	1.1156	1.1202	1.1136	1.1379	1.1551	1.1557
D	0.7216	0.7870	0.9226	1.0121	1.0600	1.0894	1.0989	1.0961	1.1141	1.1111	1.1270	1.1732
E	0.6967	0.7983	0.9225	1.0031	1.0596	1.0746	1.1042	1.1133	1.1044	1.1590	1.1728	1.1774
F	0.6882	0.7947	0.9289	1.0093	1.0604	1.0795	1.0737	1.1146	0.9687	1.1260	1.1334	1.1814
G	0.7017	0.8078	0.9246	1.0306	1.0682	1.0745	0.7771	1.1150	0.9863	0.9121	1.1146	1.1666
H	0.6926	0.8032	0.9143	1.0044	1.0368	1.0517	0.9296	1.0762	1.1026	0.8977	0.9085	1.0838

2) For each individual well, subtract the absorbance at 600 nm from the absorbance at 570 nm and average the media only control wells. The average of the media only control wells will likely be a negative number.
    Note: Wells with low conversion of PrestoBlue™ reagent will give negative values.   This is expected.
    Note: If your plate reader **did** ask for a reference or normalization wavelength, this calculation may have already been performed

570 nm-600 nm Data

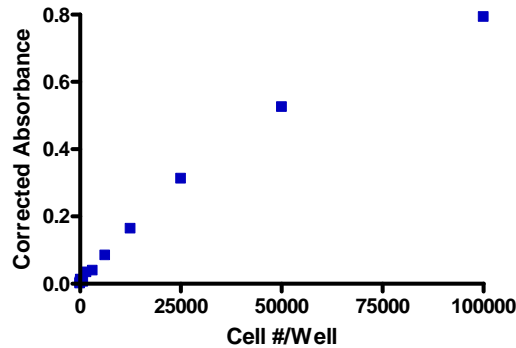| Cells/Well	100,000	50,000	25,000	12,500	6,250	3,125	1,563	781	391	195	98	Media Only
<>	1	2	3	4	5	6	7	8	9	10	11	12
A	0.5964	0.3189	0.1085	-0.0396	-0.1128	-0.1504	-0.1747	-0.1823	-0.1878	-0.1930	-0.2000	-0.1958
B	0.5802	0.3339	0.1009	-0.0352	-0.1224	-0.1635	-0.1874	-0.1964	-0.2002	-0.2027	-0.2057	-0.2016
C	0.5836	0.3354	0.1113	-0.0384	-0.1196	-0.1664	-0.1877	-0.2038	-0.2042	-0.2090	-0.2085	-0.2041
D	0.5859	0.3372	0.1277	-0.0296	-0.1205	-0.1625	-0.1897	-0.1948	-0.2069	-0.2077	-0.2036	-0.2051
E	0.5923	0.3159	0.1225	-0.0278	-0.1103	-0.1644	-0.1834	-0.1986	-0.1979	-0.2136	-0.2140	-0.2072
F	0.6020	0.3278	0.1084	-0.0396	-0.1157	-0.1637	-0.1799	-0.2000	-0.1619	-0.2070	-0.2037	-0.2045
G	0.5958	0.3230	0.1076	-0.0387	-0.1127	-0.1607	-0.0894	-0.1943	-0.1602	-0.1353	-0.1919	-0.2033
H	0.6103	0.3122	0.1160	-0.0359	-0.1050	-0.1501	-0.1346	-0.1822	-0.1929	-0.1290	-0.1333	-0.1741

Average Media Only Control Wells: -0.1995

3) Subtract the Average Control Well Value from each experimental well.
   Background Corrected Values:

Cells/Well	100,000	50,000	25,000	12,500	6,250	3,125	1,563	781	391	195	98	Media Only
<>	1	2	3	4	5	6	7	8	9	10	11	12
A	0.7959	0.5184	0.3080	0.1599	0.0867	0.0491	0.0248	0.0172	0.0117	0.0065	-0.0005	0.0037
B	0.7797	0.5334	0.3004	0.1643	0.0771	0.0360	0.0121	0.0031	-0.0007	-0.0032	-0.0062	-0.0021
C	0.7831	0.5349	0.3108	0.1611	0.0799	0.0331	0.0118	-0.0043	-0.0047	-0.0095	-0.0090	-0.0046
D	0.7854	0.5367	0.3272	0.1699	0.0790	0.0370	0.0098	0.0047	-0.0074	-0.0082	-0.0041	-0.0056
E	0.7918	0.5154	0.3220	0.1717	0.0892	0.0351	0.0161	0.0009	0.0016	-0.0141	-0.0145	-0.0077
F	0.8015	0.5273	0.3079	0.1599	0.0838	0.0358	0.0196	-0.0005	0.0376	-0.0075	-0.0042	-0.0050
G	0.7953	0.5225	0.3071	0.1608	0.0868	0.0388	0.1101	0.0052	0.0393	0.0642	0.0076	-0.0038
H	0.8098	0.5117	0.3155	0.1636	0.0945	0.0494	0.0649	0.0173	0.0066	0.0705	0.0662	0.0254
Average Corrected Values:	0.7928	0.5250	0.3123	0.1639	0.0846	0.0393	0.0336	0.0054	0.0105	0.0123	0.0044	0.0000

4) Graphing

Example data:

Calculation of the percent reduction of the PrestoBlue™ reagent.

Alternatively, the data can be expressed as a percent reduction of PrestoBlue™ reagent. In this case, the data quality remains the same, but the absorbance values are converted to a percentage of reduction of the PrestoBlue™ reagent by using the molar extinction coefficients of the oxidized and reduced forms of PrestoBlue™ reagent. The percent reduction of PrestoBlue™ reagent can be calculated from the absorbance data by using the following equation:

$$\text{Percent Reduction of PrestoBlue™ Reagent} = \frac{(O_2 \times A_1) - (O_1 \times A_2)}{(R_1 \times N_2) - (R_2 \times N_1)} \times 100$$

Where: O_1=molar extinction coefficient of oxidized PrestoBlue™ reagent at 570 nm
O_2=molar extinction coefficient of oxidized PrestoBlue™ reagent at 600 nm
R_1= molar extinction coefficient of reduced PrestoBlue™ reagent at 570 nm
R_2= molar extinction coefficient of reduced PrestoBlue™ reagent at 600 nm
A_1=absorbance of test wells at 570 nm
A_2=absorbance of test wells at 600 nm
N_1=absorbance of media only wells at 570 nm
N_2=absorbance of media only wells at 600 nm

Molar Extinction Coefficients of PrestoBlue™ Reagent

Wavelength	Reduced (R)	Oxidized (O)
570 nm	155677	80586
600 nm	14652	117216

Fitting the known constants into Equation 1, this equation now becomes:

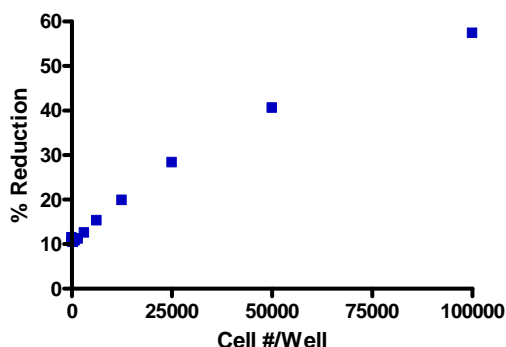% Reduction of PrestoBlue™ Reagent = $\dfrac{(117216 \times A1) - (80586 \times A2)}{(155677 \times N2) - (14652 \times N1)} \times 100$

Using the raw 570 nm and 600 nm data from page 1 as an example and fitting to this equation, the percent reduction of PrestoBlue™ reagent would be:

Percent PrestoBlue™ Reagent Reduced:

Cell #/Well	100000	50000	25000	12500	6250	3125	1563	781	391	195	98	Media Only
<>	1	2	3	4	5	6	7	8	9	10	11	12
A	57.41	40.29	28.02	19.64	15.38	13.33	11.66	11.53	11.22	11.05	10.95	11.56
B	56.91	41.12	27.62	19.83	14.74	12.44	11.13	11.05	10.60	10.68	10.43	11.52
C	56.58	41.21	28.31	19.76	15.17	12.60	11.36	10.32	10.15	10.35	10.76	11.08
D	57.26	41.15	29.36	20.23	14.87	12.56	10.85	10.43	9.97	9.85	10.49	11.40
E	57.16	39.90	28.99	20.16	15.58	12.10	11.41	10.54	10.39	10.49	10.76	11.34
F	57.66	40.66	28.14	19.46	15.22	12.25	10.98	10.47	9.94	10.22	10.62	11.62
G	57.52	40.61	27.98	20.00	15.60	12.36	10.83	10.88	10.45	10.57	11.04	11.38
H	58.34	39.75	28.35	19.62	15.45	12.60	11.00	10.88	10.70	10.69	10.63	11.62
Average % Reduction:	57.36	40.59	28.35	19.84	15.25	12.53	11.15	10.76	10.43	10.49	10.71	11.44

Graphing Percent Reduction of PrestoBlue™ Reagent data:

Technical Support

For additional assistance in using PrestoBlue™ Cell Viability Reagent, please contact the Molecular Probes technical support team. Email probestech@invitrogen.com or phone (800) 438-2209 or (541) 335-0353.