

TECHNICAL *Information*

NeA-Blue TMB Substrate (aqueous) and the addition of 1N H₂SO₄ Stop Solution

The following two charts are studies showing absorbance reading after the addition of 50 microliter and 100 microliter of 1N H₂SO₄. The absorbance readings were taken at 450nm at timed intervals after the addition of 1N H₂SO₄ to show the stability of the final color reaction with 100 microliter of TMB Substrate.

Chart 1

| Lot #: TMB01016-1-01141-A | Absorbance Readings after the addition of <u>50 microliters</u> 1N H ₂ SO ₄ Stop Solution at 450nm | | | | | | | |
|------------------------------|--|----------|----------|-----------|-----------|-----------|-----------|-----------|
| Row / Well ID | HRP - Conjugate | 1 minute | 5 minute | 10 minute | 15 minute | 20 minute | 25 minute | 30 minute |
| A | BLANK | 0.068 | 0.066 | 0.065 | 0.064 | 0.063 | 0.063 | 0.062 |
| B | 1:2 | > 3.000 | > 3.000 | 2.777 | 2.075 | 1.666 | 1.431 | 1.292 |
| C | 1:4 | 2.468 | 2.462 | 2.442 | 2.408 | 2.360 | 2.297 | 2.233 |
| D | 1:8 | 1.232 | 1.237 | 1.231 | 1.220 | 1.207 | 1.194 | 1.180 |
| E | 1:16 | 0.651 | 0.651 | 0.646 | 0.639 | 0.631 | 0.623 | 0.614 |
| F | 1:32 | 0.317 | 0.316 | 0.312 | 0.307 | 0.302 | 0.297 | 0.292 |
| G | 1:64 | 0.179 | 0.177 | 0.175 | 0.171 | 0.168 | 0.166 | 0.162 |

Chart 2

| Lot #: TMB01016-1-01141-A | Absorbance Readings after the addition of <u>100 microliters</u> 1N H ₂ SO ₄ Stop Solution at 450nm | | | | | | | |
|------------------------------|---|----------|----------|-----------|-----------|-----------|-----------|-----------|
| Row / Well ID | HRP - Conjugate | 1 minute | 5 minute | 10 minute | 15 minute | 20 minute | 25 minute | 30 minute |
| A | BLANK | 0.073 | 0.071 | 0.069 | 0.068 | 0.067 | 0.067 | 0.066 |
| B | 1:2 | >3.000 | > 3.000 | > 3.000 | > 3.000 | > 3.000 | > 3.000 | 2.671 |
| C | 1:4 | 2.122 | 2.129 | 2.123 | 2.108 | 2.090 | 2.068 | 2.044 |
| D | 1:8 | 1.090 | 1.087 | 1.081 | 1.072 | 1.063 | 1.053 | 1.042 |
| E | 1:16 | 0.615 | 0.613 | 0.607 | 0.601 | 0.595 | 0.588 | 0.580 |
| F | 1:32 | 0.340 | 0.338 | 0.334 | 0.329 | 0.325 | 0.321 | 0.316 |
| G | 1:64 | 0.189 | 0.189 | 0.186 | 0.183 | 0.181 | 0.179 | 0.176 |

Preparation of H₂SO₄ –

2 Molar (4 Normal, 4N) = 11.1 mL of concentrated H₂SO₄ to 100 mL deionized water.

1 Molar (2 Normal, 2N) = 5.5 mL concentrated H₂SO₄ to 100 mL deionized water.

0.5 Molar (1 Normal, 1N) = 2.7 mL concentrated H₂SO₄ to 100 mL deionized water.

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