RAPID EPS Evidence Sheet
—Prevention of cross contamination—

【Summary】

For plate seals, it is vital to prevent cross contamination between wells. Therefore, we evaluated the prevention level of RAPID EPS by shaking a plate sealed with EPS at 1000rpm. The absorbance and direct observation showed EPS prevented cross contamination between wells.

【Method】

The contamination prevention level was evaluated by using liquid I and liquid II, which colors after being mixed each other. These liquid were dispensed in the shaped of a checkered pattern in 96 well plates. Then, the plate was sealed with RAPID EPS, then was shaken at 1000rpm for 24 hours at 40 ℃ . The absorbance variation was measured before and after the shaking incubation.

Details of liquid were below.

Liquid I:Fe^{2+}Solution
Ferrous ammonium sulfate hexahydrate 18.0g
Ascorbic acid 2.0g
Pure water 100ml

Liquid II:Pnenanthorline Solution
1,10-phenantroline hydrate 2.0g
Sodium acetate buffer (pH 4.6) 100ml
On the occurrence of contamination and mixing between wells of liquid I and II, the red complex of $Fe^{2+}$and phenanthroline is formed under acidic condition. The absorbance of complex was detected at 540nm.Micro plate is 96 well assay plate (3881-096, material :Polystyrene, flat bottom, well volume: 0.35ml)Each liquid of 300µl was dispensed as showed below.

＜Table 1＞ **Cross position of liquid and II**

	1	2	3	4	5	6	7	8	9	10	11	12
A	I	II	I	II	I	II	I	II	I	II	I	II
B	II	I	II	I	II	I	II	I	II	I	II	I
C	I	II	I	II	I	II	I	II	I	II	I	II
D	II	I	II	I	II	I	II	I	II	I	II	I
E	I	II	I	II	I	II	I	II	I	II	I	II
F	II	I	II	I	II	I	II	I	II	I	II	I
G	I	II	I	II	I	II	I	II	I	II	I	II
H	II	I	II	I	II	I	II	I	II	I	II	I

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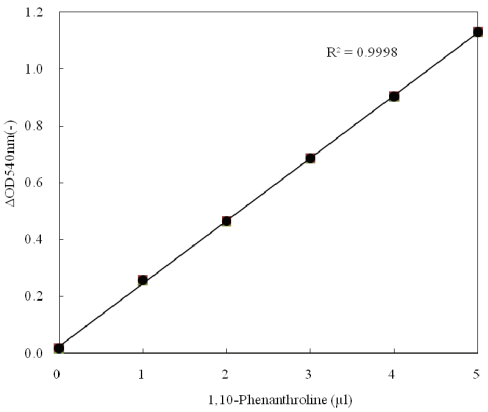
Table 2 ： Liquid color change after contamination of liquid I and II

	Liquid I Fe^{2+} Volume	Liquid II Pnenanthorline Volume
	300µl	0µl
	0µl	300µl
	300µl	1µl
	1µl	300µl
	300µl	2µl
	2µl	300µl
	300µl	3µl
	3µl	300µl
	300µl	4µl
	4µl	300µl
	300µl	5µl
	5µl	300µl

Graph 1 ： Graph for absorbance at OD540 of mixture of liquid I and II



【Evaluation of cross contamination】

The addition of 1µl of liquid II to liquid I increased absorbance by 0.2 at OD 540nm. In the case of the addition of liquid I to liquid II was calculated using molecular concentration ratio of two liquids, which revealed that approximately 0.1µl increased absorbance by 0.2 at OD 540nm.

From these results, it was concluded that contamination should have occurred when the absorbance change was more than 0.2 before and after incubation.

〖Result】

The absorbance gap between before and after shaking incubation was less than 10% of the pre-set evaluation standard above (Table 3). Then, color change was not observed. These results concluded that RAPID EPS prevented cross contamination (Figure 1). The red coloring spot observed outside wells was because of droplets of liquid I and II clinging to seals dropped on the outside wells, which was not cross contamination.

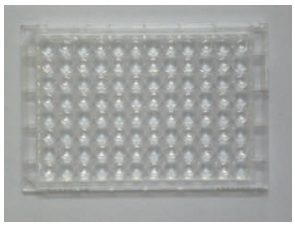
Table 3 ：RAPID EPS: Absorbance difference between before and after incubation at OD540nm

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.016	0.009	0.012	0.016	0.011	0.007	0.010	0.007	0.008	0.003	0.008	0.010
B	0.010	0.015	0.017	0.009	0.010	0.013	0.010	0.012	0.014	0.009	0.009	0.011
C	0.016	0.011	0.016	0.009	0.017	0.013	0.009	0.011	0.012	0.006	0.010	0.004
D	0.009	0.014	0.008	0.008	0.011	0.013	0.013	0.016	0.007	0.008	0.000	0.013
E	0.011	0.010	0.015	0.017	0.010	0.010	0.015	0.009	0.015	0.004	0.010	0.003
F	0.014	0.013	0.014	0.011	0.015	0.011	0.012	0.011	0.012	0.011	0.008	0.017
G	0.010	0.014	0.009	0.011	0.015	0.015	0.017	0.017	0.009	0.010	0.011	0.006
H	0.015	0.008	0.013	0.013	0.008	0.012	0.006	0.009	0.001	0.011	0.001	0.011

Figure 1 ：Coloring comparison

No cross contamination from the absorbance and direct observation

Before



After