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 EPS by shaking a plate sealed with EPS at l000 rpm. 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 EPS, then was shaken at l000 rpm for 24 hours at 40 QC . 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 l8.0 g
Ascorbic acid 2.0 g
Pure water 100 ml

Liquid II:Pnenanthorline Solution
l, l 0-phenantroline hydrate 2.0 g
Sodium acetate buffer (pH 4.6) 100 ml
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 540 nm. Micro plate is 96 well assay plate (3881-096, material :Polystyrene, flat bottom, well volume: 0.35 ml) Each liquid of 300 µl was dispensed as showed below.

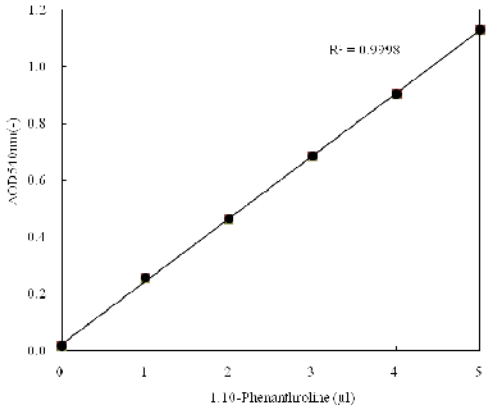
＜Table 1＞ **Cross position of liquid and II**

	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

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 1 µl of liquid II to liquid I increased absorbance by 0.2 at OD 540 nm. 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 0.1 µl increased absorbance by 0.2 at OD 540 nm.
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 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.

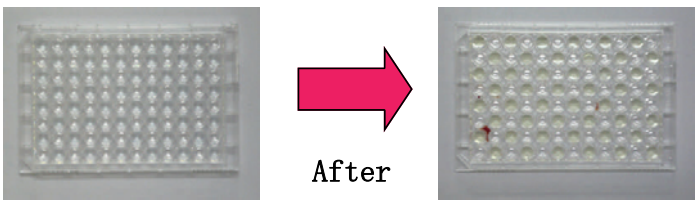
Table 3 ： EPS: Absorbance difference between before and after incubation at OD 540 nm

	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