

Appendix 8.4 Rational for the quality control acceptance ranges

- (1) The acceptance range of the absolute OD from the negative control, >0.4 was obtained from the previous Shiseido's data. The results using >0.4 showed that the SIRC-CVS:TEA test was appropriate as an alternative method for eye irritation (JaCVAM, 2011).

Table 1. Mean OD of negative control in the Shiseido's data

No.	Mean OD of the negative control (12wells)
1	0.725
2	0.648
3	0.906
4	1.085
5	0.739
6	0.582
7	0.784
8	0.731
9	0.660
10	0.731
11	0.703
12	0.648
13	0.668
14	0.670
15	0.879
16	0.965
17	0.854
18	0.976
19	0.749
20	0.961
21	0.681
22	0.617
23	0.889
24	0.648
25	1.023
26	0.987
27	0.872
28	0.822
29	0.990
30	0.658
31	0.684
32	0.578
33	0.746
34	0.654
35	0.653
36	0.649
37	0.675
38	0.933
39	1.110
40	0.958
41	0.914
42	0.883
43	0.718
44	0.923
45	0.586
46	0.870
47	0.707
48	0.862
49	0.747
50	0.714
51	0.712
52	0.728
53	0.975
54	0.748
55	0.744
56	0.857
57	0.667
58	0.736
59	0.676
60	0.806
61	0.658
62	0.813
63	0.811
64	0.630

65	0.658
66	0.646
67	0.624
68	0.583
69	0.742
70	0.686
71	0.684
72	0.780
73	0.670
74	0.874
75	0.809
76	0.798
77	0.782
78	0.725
79	0.692
80	0.716
81	0.777
82	0.811
83	0.565
84	0.775
85	0.706
86	0.723
87	0.643
88	0.689
89	0.774
90	0.703
91	0.689
92	0.701
93	0.719
94	0.731
95	0.785
96	0.821
97	0.812
98	0.695
99	0.669
100	0.736
101	0.695
102	0.739
103	0.684
104	0.715
105	0.662
106	0.705
107	0.704
108	0.566
109	0.717
110	0.668
111	0.689
112	0.749
113	0.799
114	0.634
115	0.784
116	0.668
117	0.711
118	0.691
119	0.796
120	0.630
121	0.732
122	0.735
123	0.630
124	0.699
125	0.805
126	0.719
127	0.751
128	0.645
129	0.776
130	0.719
131	0.748
132	0.728
133	0.766
134	0.781
135	0.727
136	0.662
137	0.643
138	0.647
139	0.782
140	0.617
Average	0.745
Standard deviation	0.106

Table 2. Rejected mean OD of negative control in the Shiseido's data

No.	Mean OD of the negative control (12 wells)	The reason of rejection
1	0.351	The substance, 2,4-Difluoronitrobenzene affected the negative control wells.
2	0.320	The substance, 2,4-Difluoronitrobenzene affected the negative control wells.
3	0.378	The substance, 2,4-Difluoronitrobenzene affected the negative control wells.

- (2) The acceptance range for IC₅₀ of SDS, 77.7-258.7μg/mL was obtained from mean±3SD in the previous validation study data of MHW (Tani et al, 1999). That was confirmed by the previous Shiseido's data as shown in table 3 and 4.

Table 3. IC₅₀ of SDS in the Shiseido's data

No	IC ₅₀ (μg/mL) of SDS
1	102.2
2	90.8
3	87.2
4	89.1
5	91.1
6	91.8
7	91.0
8	93.2
9	98.0
10	104.4
11	97.0
12	90.5
13	95.1
14	90.5
15	92.5
16	103.1
17	93.1
18	101.7
19	92.4
20	90.6
21	96.5
22	95.1
23	89.6
24	96.1
25	89.4
26	91.4
27	86.0
28	92.4
29	94.8
30	96.2
31	96.7
32	90.3
33	89.7
34	90.7
35	95.1
36	90.8
37	100.8
38	98.8
39	88.1
40	101.7
41	91.5
42	108.0
43	91.3
44	103.2
45	0.9

46	92.7
47	91.4
48	100.2
49	91.5
50	97.2
51	89.1
52	103.5
53	90.6
54	113.7
55	89.0
56	107.2
57	91.0
58	93.5
59	96.4
60	85.9
61	93.0
62	91.8
63	90.4
64	91.2
65	92.8
66	92.1
67	95.3
68	96.9
69	87.1
70	91.9
71	90.4
72	96.0
73	113.6
74	86.3
75	92.4
76	93.4
77	91.1
78	95.2
79	94.3
80	91.8
81	88.2
82	95.5
83	93.9
84	93.3
85	92.9
86	96.0
87	91.7
88	94.0
89	91.1
90	90.7
91	92.5
92	89.9
93	90.1
94	90.8
95	89.7
96	94.4
97	94.3
98	96.6
99	91.0
100	90.0
101	92.9
102	92.0
103	92.7
104	91.5
105	93.6
106	91.5
107	109.2
108	90.7
109	91.3
110	92.2
111	92.2
112	89.1
113	93.5
114	93.0
115	87.2
116	98.7
117	101.6
118	93.6
119	89.7

120	93.6
121	91.5
122	96.5
123	93.8
124	100.6
125	91.8
126	88.1
127	91.3
128	93.7
129	93.8
130	93.5
131	89.1
132	92.1
133	96.6
134	95.2
135	92.8
136	91.0
137	94.4
138	92.6
139	91.4
140	91.9
Average	93.1
Standard deviation	9.2

Table 4. Rejected IC₅₀ of SDS in the Shiseido's data

No.	IC ₅₀ (μg/mL) of SDS	The reason of rejection
1	63.2	Deviation of data of SDS
2	37.0	The substance, 2,4-Difluoronitrobenzene affected the other wells by volatilization
3	17.1	The substance, 2,4-Difluoronitrobenzene affected the other wells by volatilization.

- (3) The acceptance range of IC₅₀ of TEA, 1000-2500 µg/mL was obtained from the previous validation study data of MHW (Tani et al, 1999), the Shiseido's data and Phase I data of this validation.

The Shiseido's data was obtained using the acceptable range of 1000-5000 µg/mL on the basis of the validation study of MHW. The range was appropriate as shown in table 5 and 6. After the phase I study, the range was changed from 1000-5000 µg/mL to 1000-2500 µg/mL on the basis of the results as shown in table 7.

Table 5. IC₅₀ of TEA in the Shiseido's data

No	IC ₅₀ (µg/mL) of TEA
1	2164.2
2	1620.4
3	2000.5
4	1808.3
5	1675.7
6	1401.5
7	1757.2
8	1604.0
9	1044.4
10	1656.6
11	1687.6
12	1768.5
13	1940.1
14	1674.5
15	1709.2
16	1704.8
17	2228.9
18	1694.8
19	1558.9
20	1386.6
21	1868.2
22	1663.4
23	1669.9
24	1576.9
25	1932.9
26	1461.8
27	1945.1
28	1599.5
29	1424.0
30	1251.7
31	1666.2
32	1347.1
33	1012.3
34	1595.4
35	1526.8
36	1690.2
37	1501.7
38	1448.5
39	1763.3
40	1206.8
41	1773.9
42	1808.9
43	1614.6
44	1452.7
45	1435.9
46	1295.2

47	1500.2
48	1429.1
49	1525.0
50	1683.3
51	1820.5
52	1451.3
53	1349.7
54	1782.0
55	1786.8
56	1757.9
57	1664.1
58	1118.3
59	1338.9
60	1452.3
61	2145.3
62	1669.1
63	1861.3
64	1330.7
65	1770.2
66	1488.4
67	1611.9
68	1534.3
69	1550.9
70	2290.9
71	1408.8
72	1437.1
73	1260.3
74	1441.2
75	1267.2
76	1374.6
77	1695.5
78	1354.3
79	1495.1
80	1486.9
81	1339.4
82	1303.1
83	1218.0
84	1662.7
85	1484.0
86	1485.4
87	1468.0
88	1696.4
89	1531.6
90	1452.4
91	1222.7
92	1557.3
93	1737.8
94	1555.9
95	1662.5
96	1647.2
97	1706.2
98	1283.2
99	1436.5
100	1700.4
101	1446.6
102	1508.0
103	1471.9
104	2276.3
105	1545.5

106	1565.2
107	1584.5
108	1552.1
109	1413.8
110	1498.2
111	1439.4
112	1601.9
113	1622.5
114	1009.0
115	1621.0
116	1499.5
117	1464.9
118	1381.5
119	1857.2
120	1628.4
121	1403.1
122	1424.0
123	1446.3
124	1713.5
125	1781.1
126	1513.6
127	1550.3
128	1631.5
129	1341.0
130	1825.7
131	1586.1
132	1685.9
133	1576.9
134	1769.7
135	1446.2
136	1642.3
137	1549.9
Average	1575.0
Standard deviation	225.8

Table 6. Rejected IC50 of TEA in the Shiseido's data

No.	IC50 (µg/mL) of TEA	The reason of rejection
1	908.3	Deviation of data of TEA
2	603.4	The substance, 3-Chloropropionitrile affected the other wells by volatilization.
3	662.8	The substance, 2,4-Difluoronitrobenzene affected the other wells by volatilization.
4	654.0	The substance, 2,4-Difluoronitrobenzene affected the other wells by volatilization.
5	72.4	The substance, 2,4-Difluoronitrobenzene affected the other wells by volatilization.
6	127.3	The substance, 2,4-Difluoronitrobenzene affected the other wells by volatilization.

Table 7. IC50 of TEA in the three labs of the phase I

N	Mean (µg/mL)	SD
4	1382.8	33.3
4	1529.3	132.7
4	1280.8	61.34

- (4) The acceptance range of the difference between two dilution series of the substance on the plate, within $\pm 20\%$ was obtained from the previous Shiseido's data as shown in table 8 and 9.

Table 8. IC50 from two dilution series of the substance on the plate

Maximal conc.($\mu\text{g/mL}$)	IC50 ($\mu\text{g/mL}$) (1)	IC50 ($\mu\text{g/mL}$) (2)	Average IC50	Average*0.8	Average*1.2	Evaluation
5000	2979.9	2979.9	2978.4	2382.7	3574.1	Pass
5000	3442.3	3377.9	3408.2	2726.6	4089.9	Pass
5000	1879.7	2210.9	1999.0	1599.2	2398.8	Pass
5000	1491.3	1380.6	1439.6	1151.7	1727.5	Pass
5000	2377.2	3128.0	2729.0	2183.2	3274.8	Pass
5000	3627.8	3675.0	3646.0	2916.8	4375.2	Pass
500	47.3	47.0	47.2	37.7	56.6	Pass
500	50.1	50.1	50.1	40.1	60.1	Pass
5000	48.4	55.4	52.3	41.8	62.8	Pass
5000	2806.2	2576.5	2890.8	2312.7	3469.0	Pass
5000	2321.3	2490.2	2366.4	1893.2	2839.7	Pass
5000	3133.4	3351.4	3239.4	2591.5	3887.3	Pass
5000	1463.7	1408.5	1436.8	1149.5	1724.2	Pass
5000	1272.7	1355.7	1315.7	1052.5	1578.8	Pass
5000	44.1	54.1	49.1	39.3	58.9	Pass
5000	110.2	140.7	125.5	100.4	150.6	Pass
5000	56.9	49.2	53.3	42.7	64.0	Pass
5000	53.7	56.6	55.2	44.1	66.2	Pass
5000	1743.9	1569.5	1665.9	1332.7	1999.1	Pass
5000	1825.2	1613.4	1687.2	1349.8	2024.7	Pass
5000	3828.2	4046.7	3889.9	3111.9	4667.8	Pass
5000	3818.3	3812.7	3816.8	3053.4	4580.1	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	89.3	78.1	84.3	67.4	101.1	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	351.9	294.2	327.7	262.2	393.2	Pass
5000	100.2	95.0	97.7	78.2	117.3	Pass
5000	77.2	92.5	85.7	68.5	102.8	Pass
50	26.1	32.2	30.4	24.3	36.5	Pass
50	36.9	35.3	36.2	28.9	43.4	Pass
500	35.1	32.9	33.4	26.8	40.1	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	1363.4	1399.4	1381.1	1104.9	1657.4	Pass
5000	1043.2	962.5	1010.5	808.4	1212.6	Pass
5000	39.1	42.0	39.1	31.3	46.9	Pass
5000	150.7	190.3	169.6	135.7	203.6	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	392.6	406.4	399.6	319.7	479.5	Pass
5000	220.5	217.4	219.0	175.2	262.8	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	3132.5	3124.8	3126.7	2501.3	3752.0	Pass
5000	1456.4	1341.3	1399.5	1119.6	1679.4	Pass
5000	937.1	1020.8	976.2	781.0	1171.5	Pass
5000	4086.9	5000.0	4599.9	3679.9	5519.8	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	96.6	83.3	92.9	74.3	111.4	Pass
5000	79.4	73.1	75.9	60.8	91.1	Pass
5000	1868.0	2378.9	2099.4	1679.5	2519.3	Pass
5000	2268.6	2277.1	2275.0	1820.0	2730.0	Pass

5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	77.7	83.9	81.0	64.8	97.2	Pass
5000	75.6	65.8	69.7	55.7	83.6	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	402.1	340.5	371.6	297.3	446.0	Pass
5000	55.1	51.0	53.2	42.6	63.8	Pass
5000	53.0	57.5	55.5	44.4	66.6	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	3928.8	3271.3	3606.0	2884.8	4327.2	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	2186.8	2943.3	2482.3	1985.9	2978.8	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	274.0	305.1	289.3	231.4	347.1	Pass
5000	534.7	671.0	621.0	496.8	745.2	Pass
5000	755.5	751.4	753.9	603.1	904.7	Pass
5000	1032.0	859.7	969.6	775.7	1163.5	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	52.2	53.8	53.0	42.4	63.6	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	405.4	395.3	401.8	321.5	482.2	Pass
5000	412.6	340.8	386.7	309.3	464.0	Pass
5000	1789.8	1784.3	1787.0	1429.6	2144.4	Pass
5000	2664.1	2628.6	2645.0	2116.0	3174.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	832.6	819.9	827.1	661.7	992.6	Pass
5000	1081.7	943.4	1012.7	810.1	1215.2	Pass
5000	1295.1	1436.8	1347.5	1078.0	1617.0	Pass
5000	755.1	558.4	639.4	511.5	767.3	Pass
5000	749.9	820.1	785.4	628.3	942.5	Pass
5000	848.0	888.0	865.7	692.5	1038.8	Pass
5000	3116.6	3182.9	3142.8	2514.3	3771.4	Pass
5000	1281.0	1565.8	1441.1	1152.9	1729.3	Pass
5000	915.6	917.6	916.5	733.2	1099.8	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	39.1	39.1	39.1	31.3	46.9	Pass
5000	229.1	239.1	234.4	187.5	281.3	Pass
5000	239.8	243.5	241.6	193.3	290.0	Pass
5000	1481.9	1457.1	1470.1	1176.0	1764.1	Pass
5000	1409.0	1545.2	1481.1	1184.9	1777.3	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	5000.0	5000.0	5000.0	4000.0	6000.0	Pass
5000	815.0	872.4	845.1	676.1	1014.1	Pass

Table 9. Rejected IC50 from two dilution series of the substance on the plate

Maximal conc. (µg/mL)	IC50 (µg/mL) (1)	IC50 (µg/mL) (2)	Average IC50	Average*0.8	Average*1.2	Evaluation	The reason of rejection
500	46.9	32.6	40.8	32.6	49.0	Reject	The substance, 3-Chloropropionitrile affected the other wells by volatilization.
5000	104.8	75.4	97.7	78.2	117.3	Reject	The substance, 3-Chloropropionitrile affected the other wells by volatilization.
5000	148.8	90.6	98.1	78.5	117.7	Reject	Deviation of data
500	583.8	425.5	483.0	386.4	579.6	Reject	The substance, 2,4-Difluoronitrobenzene affected the other wells by volatilization .
5000	39.1	54.2	39.1	31.3	46.9	Reject	Deviation of data
5000	58.5	39.1	49.5	39.6	59.4	Reject	Deviation of data
5000	385.5	243.1	333.9	267.1	400.7	Reject	Deviation of data
5000	2112.1	5000.0	4164.6	3331.7	4997.6	Reject	Deviation of data
5000	195.2	358.3	248.8	199.0	298.6	Reject	Deviation of data
5000	963.9	2022.5	1420.0	1136.0	1704.0	Reject	Deviation of data
5000	211.9	115.9	194.1	155.3	232.9	Reject	Deviation of data
5000	126.3	664.8	150.6	120.5	180.7	Reject	Deviation of data
5000	467.5	734.2	614.0	491.2	736.8	Reject	Deviation of data

- (5) The acceptance range of the difference between left and right wells of the negative control, within $\pm 15\%$ was obtained from the previous Shiseido's data as shown in table 10 and 11.

Table 10. OD of left and right wells of the negative control on the plate

Mean OD of left wells	Mean OD of right wells	Mean OD of negative control	Mean*0.85	Mean*1.15	Evaluation
0.757	0.694	0.726	0.617	0.871	Pass
0.646	0.650	0.648	0.551	0.778	Pass
0.942	0.870	0.906	0.770	1.087	Pass
1.070	1.100	1.085	0.922	1.302	Pass
0.727	0.751	0.739	0.628	0.887	Pass
0.586	0.579	0.583	0.495	0.699	Pass
0.782	0.786	0.784	0.666	0.941	Pass
0.711	0.751	0.731	0.621	0.877	Pass
0.718	0.602	0.660	0.561	0.792	Pass
0.659	0.802	0.731	0.621	0.877	Pass
0.649	0.647	0.648	0.551	0.778	Pass
0.715	0.622	0.669	0.568	0.802	Pass
0.697	0.643	0.670	0.570	0.804	Pass
0.898	0.861	0.880	0.748	1.055	Pass

0.929	1.002	0.966	0.821	1.159	Pass
0.864	0.844	0.854	0.726	1.025	Pass
0.948	1.004	0.976	0.830	1.171	Pass
0.695	0.802	0.749	0.636	0.898	Pass
0.952	0.969	0.961	0.816	1.153	Pass
0.653	0.709	0.681	0.579	0.817	Pass
0.644	0.590	0.617	0.524	0.740	Pass
0.868	0.910	0.889	0.756	1.067	Pass
0.676	0.619	0.648	0.550	0.777	Pass
1.026	1.020	1.023	0.870	1.228	Pass
1.024	0.950	0.987	0.839	1.184	Pass
0.876	0.868	0.872	0.741	1.046	Pass
0.808	0.837	0.823	0.699	0.987	Pass
0.993	0.987	0.990	0.842	1.188	Pass
0.694	0.621	0.658	0.559	0.789	Pass
0.730	0.638	0.684	0.581	0.821	Pass
0.536	0.620	0.578	0.491	0.694	Pass
0.735	0.757	0.746	0.634	0.895	Pass
0.649	0.658	0.654	0.555	0.784	Pass
0.610	0.695	0.653	0.555	0.783	Pass
0.664	0.635	0.650	0.552	0.779	Pass
0.944	0.922	0.933	0.793	1.120	Pass
1.168	1.052	1.110	0.944	1.332	Pass
1.047	0.870	0.959	0.815	1.150	Pass
0.883	0.945	0.914	0.777	1.097	Pass
0.919	0.848	0.884	0.751	1.060	Pass
0.726	0.711	0.719	0.611	0.862	Pass
0.970	0.875	0.923	0.784	1.107	Pass
0.581	0.590	0.586	0.498	0.703	Pass
0.873	0.867	0.870	0.740	1.044	Pass
0.766	0.648	0.707	0.601	0.848	Pass
0.823	0.901	0.862	0.733	1.034	Pass
0.756	0.738	0.747	0.635	0.896	Pass
0.722	0.706	0.714	0.607	0.857	Pass
0.688	0.735	0.712	0.605	0.854	Pass
0.742	0.714	0.728	0.619	0.874	Pass
0.924	1.026	0.975	0.829	1.170	Pass
0.775	0.721	0.748	0.636	0.898	Pass
0.763	0.724	0.744	0.632	0.892	Pass
0.847	0.866	0.857	0.728	1.028	Pass
0.672	0.662	0.667	0.567	0.800	Pass
0.706	0.766	0.736	0.626	0.883	Pass
0.678	0.675	0.677	0.575	0.812	Pass
0.834	0.779	0.807	0.686	0.968	Pass
0.669	0.647	0.658	0.559	0.790	Pass
0.834	0.791	0.813	0.691	0.975	Pass
0.808	0.814	0.811	0.689	0.973	Pass
0.695	0.566	0.631	0.536	0.757	Pass
0.644	0.671	0.658	0.559	0.789	Pass
0.630	0.662	0.646	0.549	0.775	Pass
0.616	0.633	0.625	0.531	0.749	Pass
0.647	0.519	0.583	0.496	0.700	Pass
0.714	0.770	0.742	0.631	0.890	Pass
0.739	0.633	0.686	0.583	0.823	Pass
0.622	0.746	0.684	0.581	0.821	Pass
0.756	0.804	0.780	0.663	0.936	Pass
0.652	0.687	0.670	0.569	0.803	Pass
0.939	0.809	0.874	0.743	1.049	Pass
0.808	0.809	0.809	0.687	0.970	Pass
0.756	0.839	0.798	0.678	0.957	Pass
0.813	0.751	0.782	0.665	0.938	Pass
0.709	0.741	0.725	0.616	0.870	Pass
0.720	0.664	0.692	0.588	0.830	Pass
0.687	0.746	0.717	0.609	0.860	Pass
0.802	0.752	0.777	0.660	0.932	Pass
0.849	0.772	0.811	0.689	0.973	Pass
0.602	0.527	0.565	0.480	0.677	Pass
0.724	0.825	0.775	0.658	0.929	Pass
0.689	0.723	0.706	0.600	0.847	Pass
0.697	0.749	0.723	0.615	0.868	Pass
0.687	0.599	0.643	0.547	0.772	Pass
0.708	0.670	0.689	0.586	0.827	Pass
0.719	0.829	0.774	0.658	0.929	Pass
0.677	0.728	0.703	0.597	0.843	Pass

0.701	0.677	0.689	0.586	0.827	Pass
0.691	0.711	0.701	0.596	0.841	Pass
0.716	0.723	0.720	0.612	0.863	Pass
0.718	0.744	0.731	0.621	0.877	Pass
0.769	0.801	0.785	0.667	0.942	Pass
0.835	0.808	0.822	0.698	0.986	Pass
0.801	0.824	0.813	0.691	0.975	Pass
0.672	0.717	0.695	0.590	0.833	Pass
0.709	0.629	0.669	0.569	0.803	Pass
0.722	0.751	0.737	0.626	0.884	Pass
0.673	0.717	0.695	0.591	0.834	Pass
0.753	0.726	0.740	0.629	0.887	Pass
0.655	0.712	0.684	0.581	0.820	Pass
0.691	0.739	0.715	0.608	0.858	Pass
0.747	0.578	0.663	0.563	0.795	Pass
0.721	0.688	0.705	0.599	0.845	Pass
0.762	0.645	0.704	0.598	0.844	Pass
0.516	0.616	0.566	0.481	0.679	Pass
0.752	0.682	0.717	0.609	0.860	Pass
0.647	0.689	0.668	0.568	0.802	Pass
0.687	0.692	0.690	0.586	0.827	Pass
0.775	0.724	0.750	0.637	0.899	Pass
0.787	0.811	0.799	0.679	0.959	Pass
0.627	0.641	0.634	0.539	0.761	Pass
0.790	0.779	0.785	0.667	0.941	Pass
0.720	0.616	0.668	0.568	0.802	Pass
0.762	0.660	0.711	0.604	0.853	Pass
0.746	0.637	0.692	0.588	0.830	Pass
0.763	0.829	0.796	0.677	0.955	Pass
0.590	0.670	0.630	0.536	0.756	Pass
0.696	0.768	0.732	0.622	0.878	Pass
0.793	0.676	0.735	0.624	0.881	Pass
0.659	0.601	0.630	0.536	0.756	Pass
0.682	0.716	0.699	0.594	0.839	Pass
0.833	0.777	0.805	0.684	0.966	Pass
0.768	0.671	0.720	0.612	0.863	Pass
0.766	0.737	0.752	0.639	0.902	Pass
0.650	0.639	0.645	0.548	0.773	Pass
0.805	0.747	0.776	0.660	0.931	Pass
0.774	0.665	0.720	0.612	0.863	Pass
0.716	0.780	0.748	0.636	0.898	Pass
0.711	0.745	0.728	0.619	0.874	Pass
0.810	0.722	0.766	0.651	0.919	Pass
0.729	0.832	0.781	0.663	0.937	Pass
0.714	0.740	0.727	0.618	0.872	Pass
0.643	0.681	0.662	0.563	0.794	Pass
0.631	0.655	0.643	0.547	0.772	Pass
0.688	0.606	0.647	0.550	0.776	Pass
0.843	0.721	0.782	0.665	0.938	Pass
0.644	0.591	0.618	0.525	0.741	Pass

Table 11. Rejected OD of left and right wells of the negative control on the plate

OD of left wells	OD of right wells	Mean OD of negative control	Mean*0.85	Mean*1.15	Evaluation	The reason of rejection
0.474	0.932	0.703	0.598	0.808	Reject	The substance, 3-Chloropropionitrile affected the other wells by volatilization.
0.533	0.818	0.676	0.574	0.777	Reject	The substance, 2,4-Difluoronitrobenzene affected the other wells by volatilization.
0.256	0.446	0.351	0.298	0.404	Reject	The substance, 2,4-Difluoronitrobenzene affected the other wells by volatilization.
0.230	0.410	0.320	0.272	0.368	Reject	The substance, 2,4-Difluoronitrobenzene affected the other wells by volatilization.
0.116	0.639	0.378	0.321	0.434	Reject	The substance, 2,4-Difluoronitrobenzene affected the other wells by volatilization.

- (6) The acceptance range between two test results of SDS, ≥ 2 was obtained from the previous Shiseido's data as shown in table 12 and 13.

Table 12. IC50 of two test results of SDS

IC50 (μg/mL) of SDS (1)	IC50 (μg/mL) of SDS (2)	High value/low value	Evaluation
102.2	90.8	1.13	Pass
87.2	89.1	1.02	Pass
91.1	91.8	1.01	Pass
91.0	93.2	1.02	Pass
104.4	97.0	1.08	Pass
90.5	95.1	1.05	Pass
90.5	92.5	1.02	Pass
103.1	93.1	1.11	Pass
101.7	92.4	1.10	Pass
90.6	96.5	1.07	Pass
95.1	89.6	1.06	Pass
96.1	89.4	1.07	Pass
91.4	86.0	1.06	Pass
92.4	94.8	1.03	Pass
96.2	96.7	1.01	Pass
90.3	89.7	1.01	Pass
90.7	95.1	1.05	Pass
90.8	100.8	1.11	Pass
98.8	88.1	1.12	Pass
101.7	91.5	1.11	Pass
108.0	91.3	1.18	Pass
104.2	86.0	1.21	Pass
92.7	91.4	1.01	Pass
100.2	91.5	1.10	Pass
97.2	89.1	1.09	Pass
103.5	90.6	1.14	Pass
113.7	89.0	1.28	Pass
107.2	91.0	1.18	Pass
93.5	96.4	1.03	Pass
85.9	93.0	1.08	Pass
91.8	90.4	1.02	Pass
91.2	92.8	1.02	Pass
92.1	95.3	1.03	Pass
96.9	87.1	1.11	Pass
91.9	90.4	1.02	Pass
96.0	113.6	1.18	Pass
86.3	92.4	1.07	Pass
93.4	91.1	1.03	Pass
95.2	94.3	1.01	Pass
91.8	88.2	1.04	Pass
95.5	93.9	1.02	Pass
93.3	92.9	1.00	Pass
96.0	91.7	1.05	Pass
94.0	91.1	1.03	Pass
90.7	92.5	1.02	Pass
89.9	90.1	1.00	Pass
90.8	89.7	1.01	Pass
94.4	94.3	1.00	Pass
96.6	91.0	1.06	Pass
90.0	92.9	1.03	Pass
92.0	92.7	1.01	Pass
91.5	93.6	1.02	Pass
91.5	109.2	1.19	Pass
90.7	91.3	1.01	Pass
92.2	92.2	1.00	Pass
89.1	93.5	1.05	Pass
93.0	87.2	1.07	Pass
98.7	101.6	1.03	Pass
93.6	89.7	1.04	Pass
93.6	91.5	1.02	Pass
96.5	93.8	1.03	Pass
100.6	91.8	1.10	Pass
91.3	93.7	1.03	Pass
93.8	93.5	1.00	Pass
89.1	92.1	1.03	Pass
96.6	95.2	1.01	Pass
92.8	91.0	1.02	Pass
94.4	92.6	1.02	Pass
91.4	91.9	1.01	Pass

Table 13. Rejected IC50 of two test results of SDS

IC50 (μg/mL) of SDS (1)	IC50 (μg/mL) of SDS (2)	High value/low value	Evaluation	The reason of rejection
37.0	17.1	2.16	Reject	The substance, 2,4-Difluoronitrobenzene affected the other wells by volatilization.

References

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