

Appendix 8.7 Definition of chemical classes

The chemical classes were basically defined by existence of functional group in this validation. Only surfactants were classified on the basis of function in accordance with the actual condition. The information of function was obtained from International Cosmetic Ingredient Dictionary. For example, Triton X-100 was classified as surfactant, and was not classified as alcohol or alkoxylated alcohol from Chemical class.

Even if those surfactants are re-classified by chemical class such as alcohol, ether..., the predictive capacity is not changed in the study considering applicability domain. Because those molecular weights are over 180, those surfactants are not excluded in the study of predictive capacity in this validation.

Table 1 Functional group and so on for classification of chemical class

Chemical class	Functional group and so on for classification
Acrylate	Derivatives of acrylic acid ($\text{CH}_2=\text{CH}-\text{COOH}$).
Alcohol	$\text{R}-\text{OH}$
Aldehyde	$\text{R}-\text{CHO}$
Alkali	Basic ionic salt of an alkali metal or alkali earth metal chemical element
Alkanolamine	Organic compound containing both hydroxyl group ($-\text{OH}$) and amine ($-\text{NH}_2$ $-\text{NHR}$ or $-\text{NR}_2$)
Alkene	Unsaturated hydrocarbon that contains at least one carbon-carbon double bond
Amide	$-\text{CO}-\text{NH}_2$ (or $-\text{CO}-\text{NHR}$, $-\text{CO}-\text{NR}_2$)
Amine	$-\text{NH}_2$ (or $-\text{NHR}$, $-\text{NR}_2$, $-\text{NR}_3$)
Carboxylic acid (salt)	$-\text{COOH}$, $-\text{COO}^-$
Disulfide compound	$-\text{S}-\text{S}-$
Ester	$\text{R}-\text{O}-\text{CO}-\text{R}$
Ether	$\text{R}-\text{O}-\text{R}$
Fatty acid	$-\text{C}-\text{COOH}$
Halogen compound	Organic compound containing halogen
Heterocyclic compound	Cyclic compound that has atoms of at least two different elements as members of its ring(s).

Hydrocarbon	Organic compound consisting entirely of hydrogen and carbon
Inorganic salt	Salt consisting of Inorganic compound
Ketone	$R\cdot CO\cdot R$
Metacrylate	Derivative of methacrylic acid ($CH_2=CCH_3\cdot COOH$).
Nitrile compound	$R\cdot C\equiv N$
Organic salt	Salt containing an organic ion
PABA derivative	Derivative of PABA
Phenol compound	$C_6H_5\cdot OH$
Phosphorus compound	Phosphorus-containing compound
Polycyclic compound	Organic compound featuring several closed rings of atoms, primarily carbon
Polyol	Alcohol containing multiple hydroxyl groups
Quaternary ammonium compound	$\cdot NR_4^+$
Silicon compound	Compound containing silicon
Surfactant	Compound described as surfactant at “Function” of “International Cosmetic Ingredient Dictionary”. The substance that is not contained in INCI dictionary is classified by the same manner. For example, cetylpyridinium bromide was classified as surfactant on the basis of the information of cetylpyridinium chloride.
Surfonic acid	Organosulfur compounds with the general formula $RS(=O)_2\cdot OH$
Synthetic polymer	Synthetic polymer
Thiol compound	Compound containing thiol ($\cdot SH$)
Triazapentadien compound	$\cdot N=C\cdot NR\cdot C=N\cdot$

Appendix 8.8 Analysis of overlapped data of this validation and Shiseido

The comparison of data between this validation and Shiseido was performed by overlapped 21 substances, as shown in table 1 and 2. The difference of results was found in four substances, that were 2,4- dimethyl-3-pentanol, iso-octylthioglycolate, 3,3-dithiodipropionic acid and n,n-dimethylguanidine sulfate. They contained two volatile substances, 2,4- dimethyl-3-pentanol and iso-octylthioglycolate. Also, the IC₅₀s of 3,3-Dithiodipropionic acid and n,n-Dimethylguanidine sulfate were considerably near IC₅₀ of triethanolamine.

Table 1 Overlapped data of this validation and Shiseido

Substance	CAS	MW	In vitro evaluation in this validation	In vitro evaluation in Shiseido	in vivo
Ammonium nitrate	6484-52-2	80.0	P	P	P
Cyclopentanol	96-41-3	86.1	N	N	P
3-Chloropropionitrile	542-76-7	89.5	P	P	P
Toluene	108-88-3	92.1	N	N	N
2-Methyl-1-pentanol	105-30-6	102.2	N	N	P
3-Methoxy-1,2-propanediol	623-39-2	106.1	N	N	N
2,4-Dimethyl-3-pentanol	3970-62-5	116.2	N	P	N
Propylene glycol propyl ether	1569-01-3	118.2	N	N	P
iso-Propyl bromide	75-26-3	123.0	N	N	N
Potassium tetrafluoroborate	14075-53-7	125.9	N	N	N
Ethyl-2-methyl acetoacetate	609-14-3	144.2	N	N	P
1-(2-Propoxy-1-methylethoxy)-2-propanol	29911-27-1	176.3	N	N	P
iso-Octylthioglycolate	25103-09-7	204.3	N	P	N
3,3-Dithiodipropionic acid	1119-62-6	210.3	N	P	P
Hexyl cinnamic aldehyde	101-86-0	216.3	P	P	P
Triton X-100	9002-93-1	250.4	P	P	P
Isopropyl Myristate	110-27-0	270.5	N	N	N
n,n-Dimethylguanidine sulfate	598-65-2	272.3	N	P	N
Ethyl 2,6-Dichloro-5-fluoro-beta-oxo-3-pyridinepropanoate	96568-04-6	280.1	P	P	P
N-Lauroylsarcosine sodium salt	137-16-6	293.4	P	P	P
2,4,11,13-tetraazatetra (Chlorohexidine glucocinate)	18472-51-0	897.8	P	P	P

Table 2 IC50 and evaluated results of 21 substances in this validation and Shiseido

Substance	In vitro evaluation in this validation				In vitro evaluation	In vitro evaluation in Shiseido				In vitro evaluation	In vivo
	IC50 of the first measurement (µg/mL)		IC50 of the second measurement (µg/mL)			IC50 of the first measurement (µg/mL)		IC50 of the second measurement (µg/mL)			
	Substance	Triethanolamine	Substance	Triethanolamine		Substance	Triethanolamine	Substance	Triethanolamine		
Ammonium nitrate (Lab. A) (Lab. B) (Lab. C)	1342.3 1147.5 1409.6	1518.2 1414.0 1525.1	925.5 778.5 1216.4	1352.4 1184.5 1531.5	P P P	1999.0	2000.5	1439.6	1808.3	P	P
Cyclopentanol (Lab. A) (Lab. B) (Lab. C)	3074.0 1729.6 4013.2	1232.7 1392.9 1721.6	2633.5 2187.3 3593.0	1331.5 1228.0 1851.4	N N N	2684.1	1656.6	2366.4	1687.6	N	P
3-Chloropropionitrile	<39.1	1017.5	40.4	1013.8	P	47.2	1757.2	50.1	1604.0	P	P
Toluene	>5000	1090.9	>5000	1168.3	N	>5000	1349.7	>5000	1782.0	N	N
2-Methyl-1-pentanol	>2500	1353.9	>2500	1365.5	N	1665.9	1558.9	1688.2	1386.8	N	P
3-Methoxy-1,2-propane diol	>5000	1194.4	>5000	1253.6	N	>5000	1820.5	>5000	1451.3	N	N
2,4-Dimethyl-3-pentanol	>2500	1389.2	>2500	1181.2	N	1399.8	1500.2	976.2	1429.1	P	N
Propylene glycol propyl ether (Lab. A) (Lab. B) (Lab. C)	>4865.3 3561.9 >5000	1345.6 1227.5 1524.3	>3048.1 3528.2 >5000	1215.8 1248.6 1681.5	N N N	3889.9	1868.2	3816.8	1663.4	N	P
iso-Propyl bromide	>2500	1251.8	>2500	1457.5	N	>5000	1763.3	>5000	1206.8	N	N
Potassium tetrafluoroborate (Lab. A) (Lab. B) (Lab. C)	1791.6 1949.6 >5000	1362.5 1273.8 1837.1	1783.2 3630.8 >5000	1288.3 1379.6 1532.1	N N N	4595.1	1525.0	>5000	1683.3	N	N
Ethyl-2-methyl acetoacetate	>5000	1154.1	>5000	1269.4	N	2978.4	2164.2	3410.9	1620.4	N	P
1-(2-Propoxy-1-methyl ethoxy)-2-propanol (Lab. A) (Lab. B) (Lab. C)	4130.0 3188.9 >4673.4	1517.0 1320.6 1808.9	3899.0 3654.8 >5000	1452.1 1357.8 1348.5	N N N	2729.9	1675.7	3646.0	1401.5	N	P
iso-Octylthioglycolate	>2500	1628.0	>2500	1753.1	N	399.6	1614.6	219.1	1452.7	P	N
3,3-Dithiodipropionic acid (Lab. A) (Lab. C)	1864.4 1938.6	1149.4 1340.7	1696.9 1664.5	1065.1 1025.1	N N	1436.8	1940.1	1313.8	1674.5	P	P
Hexyl cinnamic aldehyde	92.1	1302.2	98.3	1534.7	P	49.1	1709.2	125.5	1704.8	P	P
Triton X-100	<39.1	1356.1	<39.1	1241.5	P	<39.1	1945.1	<39.1	1599.5	P	P
Isopropyl Myristate	>5000	1173.1	>5000	1365.7	N	>5000	1531.6	3606.0	1452.4	N	N
n,n-Dimethylguanidine sulfate (Lab. A) (Lab. B) (Lab. C)	1323.3 626.8 3464.6	1040.3 1394.2 1831.1	1653.3 535.2 2748.7	1053.7 1488.5 1108.6	N P N	1380.8	1526.8	1018.5	1690.2	P	N
Ethyl 2,6-dichloro-5-fluoro-beta-oxo-3-pyridinepropanoate (Lab. A) (Lab. B) (Lab. C)	<46.4 86.1 93.8	1226.2 1305.8 1606.1	69.9 82.8 88.1	1372.1 1145.8 1644.9	P P P	<39.1	1932.9	84.2	1461.8	P	P
N-Lauroylsarcosine sodium salt	97.5	1445.1	70.7	1194.8	P	53.3	2228.9	55.1	1694.8	P	P
2,4,11,13-tetraazatetra (Chlorohexidine glucocinate)	<39.1	1095.4	42.4	1159.1	P	<39.1	1408.8	<39.1	1437.1	P	P

Appendix 8.9 Examination of difference by lot of triethanolamine and serum

Triethanolamine from different manufacturing lots provided consistent results. Also, differences in manufacturers or production lots of serum and SDS did not have any significant effect on test results.

Result of triethanolamine (1) Phase I & IIA

IC50 (μg/mL)	Lab.	Year	Manufacturer of triethanolamine	Lot of triethanolamine	Manufacturer of serum	Lot of serum
1416.5	A	2012	Sigma-Aldrich	BCBC2078	GIBCO	909463
1338.9	A	2012	Sigma-Aldrich	BCBC2078	GIBCO	909463
1293.0	A	2012	Sigma-Aldrich	BCBC2078	GIBCO	909463
1378.3	A	2012	Sigma-Aldrich	BCBC2078	GIBCO	909463
1379.5	A	2012	Sigma-Aldrich	BCBC2078	GIBCO	909463
1338.6	A	2012	Sigma-Aldrich	BCBC2078	GIBCO	909463
1335.9	A	2012	Sigma-Aldrich	BCBC2078	GIBCO	909463
1396.0	A	2012	Sigma-Aldrich	BCBC2078	GIBCO	909463
1439.1	A	2012	Sigma-Aldrich	BCBC2078	GIBCO	909463
1455.5	A	2012	Sigma-Aldrich	BCBC2078	GIBCO	909463
1389.7	A	2012	Sigma-Aldrich	BCBC2078	GIBCO	909463
1432.2	A	2012	Sigma-Aldrich	BCBC2078	GIBCO	909463
1777.0	B	2012	Wako	DCCK2809	Nichirei	9E0887
1117.4	B	2012	Wako	DCCK2809	Nichirei	9E0887
1760.5	B	2012	Wako	DCCK2809	Nichirei	9E0887
1543.5	B	2012	Wako	DCCK2809	Nichirei	9E0887
1602.9	B	2012	Wako	DCCK2809	Nichirei	9E0887
1593.0	B	2012	Wako	DCCK2809	Nichirei	9E0887
1138.6	B	2012	Wako	DCCK2809	Nichirei	9E0887
1213.5	B	2012	Wako	DCCK2809	Nichirei	9E0887
1666.0	B	2012	Wako	DCCK2809	Nichirei	9E0887
1559.9	B	2012	Wako	DCCK2809	Nichirei	9E0887
1700.2	B	2012	Wako	DCCK2809	Nichirei	9E0887
1679.4	B	2012	Wako	DCCK2809	Nichirei	9E0887
1587.2	C #	2012	Wako	DCCK2809	Nichirei	9E0887
1036.4	C #	2012	Wako	DCCK2809	Nichirei	9E0887
1079.8	C #	2012	Wako	DCCK2809	Nichirei	9E0887
1392.7	C #	2012	Wako	DCCK2809	Nichirei	9E0887
1064.5	C #	2012	Wako	DCCK2809	Nichirei	9E0887
1337.7	C #	2012	Wako	DCCK2809	Nichirei	9E0887
1193.0	C #	2012	Wako	DCCK2809	Nichirei	9E0887
1373.6	C #	2012	Wako	DCCK2809	Nichirei	9E0887
1545.9	C #	2012	Wako	DCCK2809	Nichirei	9E0887
1117.8	C #	2012	Wako	DCCK2809	Nichirei	9E0887
1468.5	C #	2012	Wako	DCCK2809	Nichirei	9E0887
1172.0	C #	2012	Wako	DCCK2809	Nichirei	9E0887

IC50 (μg/mL)	Lab.	Year	Manufacturer of triethanolamine	Lot of triethanolamine	Manufacturer of serum	Lot of serum
1676.6	A	2012	Wako	DCCK3718	GIBCO	1073767
1461.0	A	2012	Wako	DCCK3718	GIBCO	1073767
1298.7	A	2012	Wako	DCCK3718	GIBCO	1073767
1078.8	A	2012	Wako	DCCK3718	GIBCO	1073767
1006.3	A	2012	Wako	DCCK3718	GIBCO	1073767
1621.7	A	2012	Wako	DCCK3718	GIBCO	1073767
1802.0	A	2012	Wako	DCCK3718	GIBCO	1073767
1524.8	A	2012	Wako	DCCK3718	GIBCO	1073767
1440.4	A	2012	Wako	DCCK3718	GIBCO	1073767
1762.6	A	2012	Wako	DCCK3718	GIBCO	1073767
1590.1	A	2012	Wako	DCCK3718	GIBCO	1073767
1454.3	A	2012	Wako	DCCK3718	GIBCO	1073767
1541.3	A	2012	Wako	DCCK3718	GIBCO	1073767
1067.9	A	2012	Wako	DCCK3718	GIBCO	1073767
1235.4	A	2012	Wako	DCCK3718	GIBCO	1073767
1702.5	A	2012	Wako	DCCK3718	GIBCO	1073767
1380.5	A	2012	Wako	DCCK3718	GIBCO	1073767
1069.8	A	2012	Wako	DCCK3718	GIBCO	1073767
1484.8	A	2012	Wako	DCCK3718	GIBCO	1073767
1355.0	A	2012	Wako	DCCK3718	GIBCO	1073767
1711.3	A	2012	Wako	DCCK3718	GIBCO	1073767
1704.7	A	2012	Wako	DCCK3718	GIBCO	1073767
1040.1	A	2012	Wako	DCCK3718	GIBCO	1073767
1611.6	A	2012	Wako	DCCK3718	GIBCO	1073767
1715.7	A	2012	Wako	DCCK3718	GIBCO	1073767
1511.3	A	2012	Wako	DCCK3718	GIBCO	1073767
1313.2	A	2012	Wako	DCCK3718	GIBCO	1073767
1708.9	A	2012	Wako	DCCK3718	GIBCO	1073767
1741.3	A	2012	Wako	DCCK3718	GIBCO	1073767
1104.5	A	2012	Wako	DCCK3718	GIBCO	1073767
1616.2	A	2012	Wako	DCCK3718	GIBCO	1073767
1054.6	A	2012	Wako	DCCK3718	GIBCO	1073767
1386.5	A	2012	Wako	DCCK3718	GIBCO	1073767
1750.1	A	2012	Wako	DCCK3718	GIBCO	1073767
1468.9	A	2012	Wako	DCCK3718	GIBCO	1073767
1101.4	A	2012	Wako	DCCK3718	GIBCO	1073767
1394.3	A	2012	Wako	DCCK3718	GIBCO	1073767
1503.8	A	2012	Wako	DCCK3718	GIBCO	1073767
1189.3	A	2012	Wako	DCCK3718	GIBCO	1073767
1635.4	A	2012	Wako	DCCK3718	GIBCO	1073767
1029.0	A	2012	Wako	DCCK3718	GIBCO	1073767
1200.5	A	2012	Wako	DCCK3718	GIBCO	1073767
1607.7	A	2012	Wako	DCCK3718	GIBCO	1073767
1337.4	A	2012	Wako	DCCK3718	GIBCO	1073767
1080.5	A	2012	Wako	DCCK3718	GIBCO	1073767

Result of triethanolamine (2) Phase IIA

IC50 (μg/mL)	Lab.	Year	Manufaturer of triethanolamine	Lot of triethanolamine	Manufaturer of serum	Lot of serum
1153.5	B	2012	Wako	DCCK3718	GIBCO	1073767
1575.0	B	2012	Wako	DCCK3718	GIBCO	1073767
1159.3	B	2012	Wako	DCCK3718	GIBCO	1073767
1282.0	B	2012	Wako	DCCK3718	GIBCO	1073767
1388.1	B	2012	Wako	DCCK3718	GIBCO	1073767
1311.0	B	2012	Wako	DCCK3718	GIBCO	1073767
1228.9	B	2012	Wako	DCCK3718	GIBCO	1073767
1267.0	B	2012	Wako	DCCK3718	GIBCO	1073767
1038.0	B	2012	Wako	DCCK3718	GIBCO	1073767
1401.0	B	2012	Wako	DCCK3718	GIBCO	1073767
1558.4	B	2012	Wako	DCCK3718	GIBCO	1073767
1247.7	B	2012	Wako	DCCK3718	GIBCO	1073767
1497.4	B	2012	Wako	DCCK3718	GIBCO	1073767
1130.8	B	2012	Wako	DCCK3718	GIBCO	1073767
1364.3	B	2012	Wako	DCCK3718	GIBCO	1073767
1231.5	B	2012	Wako	DCCK3718	GIBCO	1073767
1314.4	B	2012	Wako	DCCK3718	GIBCO	1073767
1276.2	B	2012	Wako	DCCK3718	GIBCO	1073767
1279.2	B	2012	Wako	DCCK3718	GIBCO	1073767
1596.1	B	2012	Wako	DCCK3718	GIBCO	1073767
1086.4	B	2012	Wako	DCCK3718	GIBCO	1073767
1450.8	B	2012	Wako	DCCK3718	GIBCO	1073767
1046.0	B	2012	Wako	DCCK3718	GIBCO	1073767
1576.7	B	2012	Wako	DCCK3718	GIBCO	1073767
1218.0	B	2012	Wako	DCCK3718	GIBCO	1073767
1345.4	B	2012	Wako	DCCK3718	GIBCO	1073767
1307.4	B	2012	Wako	DCCK3718	GIBCO	1073767
1259.8	B	2012	Wako	DCCK3718	GIBCO	1073767
1701.7	B	2012	Wako	DCCK3718	GIBCO	1073767
1280.4	B	2012	Wako	DCCK3718	GIBCO	1073767
1048.7	B	2012	Wako	DCCK3718	GIBCO	1073767
1119.0	B	2012	Wako	DCCK3718	GIBCO	1073767
1385.7	B	2012	Wako	DCCK3718	GIBCO	1073767
1286.4	B	2012	Wako	DCCK3718	GIBCO	1073767
1431.1	B	2012	Wako	DCCK3718	GIBCO	1073767
1170.0	B	2012	Wako	DCCK3718	GIBCO	1073767
1261.3	B	2012	Wako	DCCK3718	GIBCO	1073767
1556.7	B	2012	Wako	DCCK3718	GIBCO	1073767
1003.5	B	2012	Wako	DCCK3718	GIBCO	1073767
1449.2	B	2012	Wako	DCCK3718	GIBCO	1073767
1344.8	B	2012	Wako	DCCK3718	GIBCO	1073767
1344.8	B	2012	Wako	DCCK3718	GIBCO	1073767
1260.4	B	2012	Wako	DCCK3718	GIBCO	1073767
1232.7	B	2012	Wako	DCCK3718	GIBCO	1073767
1276.2	B	2012	Wako	DCCK3718	GIBCO	1073767

IC50 (μg/mL)	Lab.	Year	Manufaturer of triethanolamine	Lot of triethanolamine	Manufaturer of serum	Lot of serum
1774.8	C	2012	Wako	DCCK3718	GIBCO	1073767
1411.5	C	2012	Wako	DCCK3718	GIBCO	1073767
1350.2	C	2012	Wako	DCCK3718	GIBCO	1073767
1430.9	C	2012	Wako	DCCK3718	GIBCO	1073767
1570.4	C	2012	Wako	DCCK3718	GIBCO	1073767
1359.4	C	2012	Wako	DCCK3718	GIBCO	1073767
1373.3	C	2012	Wako	DCCK3718	GIBCO	1073767
1051.3	C	2012	Wako	DCCK3718	GIBCO	1073767
1488.0	C	2012	Wako	DCCK3718	GIBCO	1073767
1725.7	C	2012	Wako	DCCK3718	GIBCO	1073767
1721.0	C	2012	Wako	DCCK3718	GIBCO	1073767
1818.3	C	2012	Wako	DCCK3718	GIBCO	1073767
1054.3	C	2012	Wako	DCCK3718	GIBCO	1073767
1263.3	C	2012	Wako	DCCK3718	GIBCO	1073767
1278.5	C	2012	Wako	DCCK3718	GIBCO	1073767
1439.3	C	2012	Wako	DCCK3718	GIBCO	1073767
1594.3	C	2012	Wako	DCCK3718	GIBCO	1073767
1542.3	C	2012	Wako	DCCK3718	GIBCO	1073767
1780.3	C	2012	Wako	DCCK3718	GIBCO	1073767
1696.0	C	2012	Wako	DCCK3718	GIBCO	1073767
1950.3	C	2012	Wako	DCCK3718	GIBCO	1073767
1405.1	C	2012	Wako	DCCK3718	GIBCO	1073767
1303.2	C	2012	Wako	DCCK3718	GIBCO	1073767
1337.3	C	2012	Wako	DCCK3718	GIBCO	1073767
1182.2	C	2012	Wako	DCCK3718	GIBCO	1073767
1481.0	C	2012	Wako	DCCK3718	GIBCO	1073767
1353.4	C	2012	Wako	DCCK3718	GIBCO	1073767
1556.4	C	2012	Wako	DCCK3718	GIBCO	1073767
1433.1	C	2012	Wako	DCCK3718	GIBCO	1073767
1585.8	C	2012	Wako	DCCK3718	GIBCO	1073767
1564.9	C	2012	Wako	DCCK3718	GIBCO	1073767
1512.8	C	2012	Wako	DCCK3718	GIBCO	1073767
1516.8	C	2012	Wako	DCCK3718	GIBCO	1073767
1595.8	C	2012	Wako	DCCK3718	GIBCO	1073767
1522.1	C	2012	Wako	DCCK3718	GIBCO	1073767
1406.5	C	2012	Wako	DCCK3718	GIBCO	1073767
1638.7	C	2012	Wako	DCCK3718	GIBCO	1073767
1895.5	C	2012	Wako	DCCK3718	GIBCO	1073767
1977.1	C	2012	Wako	DCCK3718	GIBCO	1073767
1566.5	C	2012	Wako	DCCK3718	GIBCO	1073767
1590.9	C	2012	Wako	DCCK3718	GIBCO	1073767
1439.0	C	2012	Wako	DCCK3718	GIBCO	1073767
1071.9	C	2012	Wako	DCCK3718	GIBCO	1073767
1317.7	C	2012	Wako	DCCK3718	GIBCO	1073767
1404.2	C	2012	Wako	DCCK3718	GIBCO	1073767

Result of triethanolamine (3) Others

IC50 (μg/mL)	Lab.	Year	Manufacturer of triethanolamine	Lot of triethanolamine	Manufacturer of serum	Lot of serum
1540.0	MHW-B	1995	Kanto	611E1858	GIBCO ^S	30P1033
1320.0	MHW-B	1995	Kanto	611E1858	GIBCO ^S	30P1033
1850.0	MHW-C	1995	Kanto	611E1858	GIBCO ^S	30P1033
1650.0	MHW-C	1995	Kanto	611E1858	GIBCO ^S	30P1033
1910.0	MHW-D	1995	Kanto	611E1858	GIBCO ^S	30P1033
2075.0	MHW-D	1995	Kanto	611E1858	GIBCO ^S	30P1033
3200.0	MHW-E	1995	Kanto	611E1858	GIBCO ^S	30P1033
4500.0	MHW-E	1995	Kanto	611E1858	GIBCO ^S	30P1033
1580.0	MHW-A(Shiseido)	1995	Kanto	611E1858	GIBCO ^S	30P1033
1300.0	MHW-A(Shiseido)	1995	Kanto	611E1858	GIBCO ^S	30P1033
2164.2	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
2000.5	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1675.7	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1757.2	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1656.6	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1940.1	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1709.2	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
2228.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1558.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1868.2	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1669.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1932.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1945.1	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1424.0	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1666.2	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1526.8	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1501.7	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1763.3	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1773.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1614.6	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1435.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1500.2	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1525.0	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1820.5	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1349.7	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1786.8	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1664.1	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1338.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
2145.3	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1861.3	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1770.2	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1611.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1550.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1408.8	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1260.3	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1267.2	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1695.5	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1495.1	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1339.4	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1218.0	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1484.0	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1468.0	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1531.6	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1222.7	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1737.8	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1662.5	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1706.2	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1436.5	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1446.6	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1471.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1545.5	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1584.5	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1413.8	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1439.4	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1622.5	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1621.0	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1464.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1857.2	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1403.1	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1713.5	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1513.6	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1631.5	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1825.7	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1685.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1769.7	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1642.3	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML

IC50 (μg/mL)	Lab.	Year	Manufacturer of triethanolamine	Lot of triethanolamine	Manufacturer of serum	Lot of serum
1620.4	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1808.3	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1401.5	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1604.0	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1687.6	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1674.5	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1704.8	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1694.8	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1386.8	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1663.4	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1576.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1461.8	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1599.5	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1251.7	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1347.1	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1690.2	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1448.5	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1206.8	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1808.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1452.7	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1295.2	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1429.1	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1683.3	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1451.3	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1782.0	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1757.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1118.3	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1452.3	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1669.1	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1330.7	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1488.4	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1534.3	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
2290.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1437.1	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1441.2	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1374.6	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1354.3	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1486.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1303.1	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1662.7	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1485.4	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1696.4	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1452.4	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1557.3	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1555.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1647.2	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1283.2	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1700.4	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1508.0	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
2276.3	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1565.2	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1552.1	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1498.2	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1601.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1009.0	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1499.5	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1381.5	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1628.4	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1424.0	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1781.1	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1550.3	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1341.0	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1586.1	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1576.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1446.2	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1549.9	Shiseido	2009	Kanto	810W1077	JRH	12603C-500ML
1012.3	Shiseido	2010	Kanto	810W1077	JRH	12603C-500ML
1595.4	Shiseido	2010	Kanto	810W1077	JRH	12603C-500ML

JRHH:JRHH Bioscience

Kanto:Kanto Chemical CO., INC.

Nichirei:Nichirei Biosciences Inc.

Wako : Wako Pure Chemical Industries, Ltd.

#:Retest

S:Calf serum was obtained from GIBCO Laboratories (NY, USA)

Difference by manufacturer and lot of TEA

Manufacturer of TEA	Lot of TEA	n	IC50 (µg/mL)	
			Average	SD
Wako	DCK2809	24	1405.0	247.2
Wako	DCK3718	135	1408.5	224.4
Sigma-Aldrich	BCBC2078	12	1382.8	49.016
Kanto	810W1077	134	1578.5	222.7
Kanto	611E1858	10	2092.5	1005.7

Other difference of test condition
-
Lab., Lot of serum
Lab., Manufacturer of serum
Lab., Manufacturer of serum
Lab., Type of serum (Calf serum)

Difference by manufacturer and lot of serum

Manufacturer of serum	Lot of serum	n	IC50 (µg/mL)	
			Average	SD
GIBCO	909463	12	1382.8	49.0
GIBCO	1073767	135	1408.5	224.4
Nichirei	9E0887	24	1405.0	247.19
JRH	810W1077	134	1578.5	222.7
GIBCO [®] (Calf serum)	30P1033	10	2092.5	1005.7

Other difference of test condition
-
Lab., Lot of TEA
Lab., Lot of TEA
Lab., Manufacturer of TEA
Lab.(5labs), Manufacturer of TEA

Result of positive control (SDS) (1) Phase I & IIA

IC50 (μg/mL)	Lab.	Year	Manufacturer of SDS	Lot of SDS	Manufacturer of Serum	Lot of serum
83.4	A	2012	Wako	SDF8154	GIBCO	909463
81.0	A	2012	Wako	SDF8154	GIBCO	909463
83.3	A	2012	Wako	SDF8154	GIBCO	909463
80.2	A	2012	Wako	SDF8154	GIBCO	909463
80.2	A	2012	Wako	SDF8154	GIBCO	909463
80.3	A	2012	Wako	SDF8154	GIBCO	909463
93.9	A	2012	Wako	SDF8154	GIBCO	909463
87.0	A	2012	Wako	SDF8154	GIBCO	909463
79.2	A	2012	Wako	SDF8154	GIBCO	909463
78.2	A	2012	Wako	SDF8154	GIBCO	909463
78.3	A	2012	Wako	SDF8154	GIBCO	909463
79.0	A	2012	Wako	SDF8154	GIBCO	909463
83.5	B	2012	Wako	SDF8154	Nichirei	9E0887
86.3	B	2012	Wako	SDF8154	Nichirei	9E0887
91.8	B	2012	Wako	SDF8154	Nichirei	9E0887
88.7	B	2012	Wako	SDF8154	Nichirei	9E0887
79.3	B	2012	Wako	SDF8154	Nichirei	9E0887
86.0	B	2012	Wako	SDF8154	Nichirei	9E0887
89.9	B	2012	Wako	SDF8154	Nichirei	9E0887
87.3	B	2012	Wako	SDF8154	Nichirei	9E0887
88.6	B	2012	Wako	SDF8154	Nichirei	9E0887
85.0	B	2012	Wako	SDF8154	Nichirei	9E0887
87.0	B	2012	Wako	SDF8154	Nichirei	9E0887
90.5	B	2012	Wako	SDF8154	Nichirei	9E0887
81.8	C #	2012	Wako	SDF8154	Nichirei	9E0887
80.8	C #	2012	Wako	SDF8154	Nichirei	9E0887
86.9	C #	2012	Wako	SDF8154	Nichirei	9E0887
83.3	C #	2012	Wako	SDF8154	Nichirei	9E0887
85.4	C #	2012	Wako	SDF8154	Nichirei	9E0887
91.1	C #	2012	Wako	SDF8154	Nichirei	9E0887
81.5	C #	2012	Wako	SDF8154	Nichirei	9E0887
93.8	C #	2012	Wako	SDF8154	Nichirei	9E0887
77.9	C #	2012	Wako	SDF8154	Nichirei	9E0887
80.5	C #	2012	Wako	SDF8154	Nichirei	9E0887
84.2	C #	2012	Wako	SDF8154	Nichirei	9E0887
87.5	C #	2012	Wako	SDF8154	Nichirei	9E0887

IC50 (μg/mL)	Lab.	Year	Manufacturer of SDS	Lot of SDS	Manufacturer of Serum	Lot of serum
82.1	A	2012	Wako	SDF8154	GIBCO	1073767
88.7	A	2012	Wako	SDF8154	GIBCO	1073767
86.7	A	2012	Wako	SDF8154	GIBCO	1073767
88.8	A	2012	Wako	SDF8154	GIBCO	1073767
85.7	A	2012	Wako	SDF8154	GIBCO	1073767
90.3	A	2012	Wako	SDF8154	GIBCO	1073767
89.4	A	2012	Wako	SDF8154	GIBCO	1073767
86.3	A	2012	Wako	SDF8154	GIBCO	1073767
88.0	A	2012	Wako	SDF8154	GIBCO	1073767
80.9	A	2012	Wako	SDF8154	GIBCO	1073767
88.4	A	2012	Wako	SDF8154	GIBCO	1073767
84.3	A	2012	Wako	SDF8154	GIBCO	1073767
94.3	A	2012	Wako	SDF8154	GIBCO	1073767
86.2	A	2012	Wako	SDF8154	GIBCO	1073767
87.4	A	2012	Wako	SDF8154	GIBCO	1073767
86.7	A	2012	Wako	SDF8154	GIBCO	1073767
86.4	A	2012	Wako	SDF8154	GIBCO	1073767
91.5	A	2012	Wako	SDF8154	GIBCO	1073767
87.7	A	2012	Wako	SDF8154	GIBCO	1073767
86.4	A	2012	Wako	SDF8154	GIBCO	1073767
84.5	A	2012	Wako	SDF8154	GIBCO	1073767
89.1	A	2012	Wako	SDF8154	GIBCO	1073767
85.7	A	2012	Wako	SDF8154	GIBCO	1073767
91.4	A	2012	Wako	SDF8154	GIBCO	1073767
87.9	A	2012	Wako	SDF8154	GIBCO	1073767
90.8	A	2012	Wako	SDF8154	GIBCO	1073767
87.3	A	2012	Wako	SDF8154	GIBCO	1073767
88.0	A	2012	Wako	SDF8154	GIBCO	1073767
87.5	A	2012	Wako	SDF8154	GIBCO	1073767
92.6	A	2012	Wako	SDF8154	GIBCO	1073767
90.4	A	2012	Wako	SDF8154	GIBCO	1073767
90.1	A	2012	Wako	SDF8154	GIBCO	1073767
91.7	A	2012	Wako	SDF8154	GIBCO	1073767
88.3	A	2012	Wako	SDF8154	GIBCO	1073767
87.0	A	2012	Wako	SDF8154	GIBCO	1073767
84.0	A	2012	Wako	SDF8154	GIBCO	1073767
81.9	A	2012	Wako	SDF8154	GIBCO	1073767
86.8	A	2012	Wako	SDF8154	GIBCO	1073767
85.2	A	2012	Wako	SDF8154	GIBCO	1073767
87.7	A	2012	Wako	SDF8154	GIBCO	1073767
87.5	A	2012	Wako	SDF8154	GIBCO	1073767
89.4	A	2012	Wako	SDF8154	GIBCO	1073767
89.4	A	2012	Wako	SDF8154	GIBCO	1073767
79.6	A	2012	Wako	SDF8154	GIBCO	1073767
87.5	A	2012	Wako	SDF8154	GIBCO	1073767

Result of positive control (SDS) (2) Phase IIA

IC50 (μg/mL)	Lab.	Year	Manufacturer of SDS	Lot of SDS	Manufacturer of Serum	Lot of serum
89.9	B	2012	Wako	SDF8154	GIBCO	1073767
91.8	B	2012	Wako	SDF8154	GIBCO	1073767
84.6	B	2012	Wako	SDF8154	GIBCO	1073767
95.8	B	2012	Wako	SDF8154	GIBCO	1073767
92.1	B	2012	Wako	SDF8154	GIBCO	1073767
93.4	B	2012	Wako	SDF8154	GIBCO	1073767
91.9	B	2012	Wako	SDF8154	GIBCO	1073767
91.5	B	2012	Wako	SDF8154	GIBCO	1073767
92.1	B	2012	Wako	SDF8154	GIBCO	1073767
92.3	B	2012	Wako	SDF8154	GIBCO	1073767
92.9	B	2012	Wako	SDF8154	GIBCO	1073767
84.6	B	2012	Wako	SDF8154	GIBCO	1073767
95.7	B	2012	Wako	SDF8154	GIBCO	1073767
100.0	B	2012	Wako	SDF8154	GIBCO	1073767
93.0	B	2012	Wako	SDF8154	GIBCO	1073767
91.7	B	2012	Wako	SDF8154	GIBCO	1073767
93.0	B	2012	Wako	SDF8154	GIBCO	1073767
92.2	B	2012	Wako	SDF8154	GIBCO	1073767
91.8	B	2012	Wako	SDF8154	GIBCO	1073767
94.0	B	2012	Wako	SDF8154	GIBCO	1073767
86.9	B	2012	Wako	SDF8154	GIBCO	1073767
93.5	B	2012	Wako	SDF8154	GIBCO	1073767
95.0	B	2012	Wako	SDF8154	GIBCO	1073767
91.0	B	2012	Wako	SDF8154	GIBCO	1073767
92.9	B	2012	Wako	SDF8154	GIBCO	1073767
94.6	B	2012	Wako	SDF8154	GIBCO	1073767
89.6	B	2012	Wako	SDF8154	GIBCO	1073767
95.3	B	2012	Wako	SDF8154	GIBCO	1073767
94.9	B	2012	Wako	SDF8154	GIBCO	1073767
89.4	B	2012	Wako	SDF8154	GIBCO	1073767
89.2	B	2012	Wako	SDF8154	GIBCO	1073767
97.3	B	2012	Wako	SDF8154	GIBCO	1073767
91.1	B	2012	Wako	SDF8154	GIBCO	1073767
91.2	B	2012	Wako	SDF8154	GIBCO	1073767
91.8	B	2012	Wako	SDF8154	GIBCO	1073767
89.4	B	2012	Wako	SDF8154	GIBCO	1073767
90.9	B	2012	Wako	SDF8154	GIBCO	1073767
94.2	B	2012	Wako	SDF8154	GIBCO	1073767
80.2	B	2012	Wako	SDF8154	GIBCO	1073767
92.3	B	2012	Wako	SDF8154	GIBCO	1073767
86.5	B	2012	Wako	SDF8154	GIBCO	1073767
86.5	B	2012	Wako	SDF8154	GIBCO	1073767
86.1	B	2012	Wako	SDF8154	GIBCO	1073767
92.5	B	2012	Wako	SDF8154	GIBCO	1073767
92.2	B	2012	Wako	SDF8154	GIBCO	1073767

IC50 (μg/mL)	Lab.	Year	Manufacturer	Lot of SDS	Manufacturer of Serum	Lot of serum
92.8	C	2012	Wako	SDF8154	GIBCO	1073767
83.2	C	2012	Wako	SDF8154	GIBCO	1073767
77.7	C	2012	Wako	SDF8154	GIBCO	1073767
89.5	C	2012	Wako	SDF8154	GIBCO	1073767
89.1	C	2012	Wako	SDF8154	GIBCO	1073767
86.6	C	2012	Wako	SDF8154	GIBCO	1073767
86.8	C	2012	Wako	SDF8154	GIBCO	1073767
80.7	C	2012	Wako	SDF8154	GIBCO	1073767
87.0	C	2012	Wako	SDF8154	GIBCO	1073767
96.1	C	2012	Wako	SDF8154	GIBCO	1073767
93.4	C	2012	Wako	SDF8154	GIBCO	1073767
91.5	C	2012	Wako	SDF8154	GIBCO	1073767
95.3	C	2012	Wako	SDF8154	GIBCO	1073767
93.2	C	2012	Wako	SDF8154	GIBCO	1073767
88.9	C	2012	Wako	SDF8154	GIBCO	1073767
92.6	C	2012	Wako	SDF8154	GIBCO	1073767
84.1	C	2012	Wako	SDF8154	GIBCO	1073767
91.7	C	2012	Wako	SDF8154	GIBCO	1073767
90.7	C	2012	Wako	SDF8154	GIBCO	1073767
88.9	C	2012	Wako	SDF8154	GIBCO	1073767
92.3	C	2012	Wako	SDF8154	GIBCO	1073767
94.5	C	2012	Wako	SDF8154	GIBCO	1073767
90.4	C	2012	Wako	SDF8154	GIBCO	1073767
84.3	C	2012	Wako	SDF8154	GIBCO	1073767
85.9	C	2012	Wako	SDF8154	GIBCO	1073767
88.0	C	2012	Wako	SDF8154	GIBCO	1073767
85.9	C	2012	Wako	SDF8154	GIBCO	1073767
81.9	C	2012	Wako	SDF8154	GIBCO	1073767
79.6	C	2012	Wako	SDF8154	GIBCO	1073767
79.5	C	2012	Wako	SDF8154	GIBCO	1073767
78.0	C	2012	Wako	SDF8154	GIBCO	1073767
85.9	C	2012	Wako	SDF8154	GIBCO	1073767
92.1	C	2012	Wako	SDF8154	GIBCO	1073767
88.3	C	2012	Wako	SDF8154	GIBCO	1073767
84.0	C	2012	Wako	SDF8154	GIBCO	1073767
87.4	C	2012	Wako	SDF8154	GIBCO	1073767
98.9	C	2012	Wako	SDF8154	GIBCO	1073767
95.8	C	2012	Wako	SDF8154	GIBCO	1073767
92.9	C	2012	Wako	SDF8154	GIBCO	1073767
90.9	C	2012	Wako	SDF8154	GIBCO	1073767
94.3	C	2012	Wako	SDF8154	GIBCO	1073767
87.6	C	2012	Wako	SDF8154	GIBCO	1073767
93.3	C	2012	Wako	SDF8154	GIBCO	1073767
83.0	C	2012	Wako	SDF8154	GIBCO	1073767
97.7	C	2012	Wako	SDF8154	GIBCO	1073767

IC50 (μg/mL)	Lab.	Year	Manufacturer	Lot.	Manufacturer of serum	Lot of serum
168	MHW-B	1994	Nikko	2802	GIBCO#	45K4613
176	MHW-B	1994	Nikko	2802	GIBCO#	45K4613
172	MHW-B	1994	Nikko	2802	GIBCO#	45K4613
117	MHW-C	1994	Nikko	2802	GIBCO#	45K4613
117	MHW-C	1994	Nikko	2802	GIBCO#	45K4613
117	MHW-C	1994	Nikko	2802	GIBCO#	45K4613
190	MHW-D	1994	Nikko	2802	GIBCO#	45K4613
190	MHW-D	1994	Nikko	2802	GIBCO#	45K4613
187	MHW-D	1994	Nikko	2802	GIBCO#	45K4613
201	MHW-E	1994	Nikko	2802	GIBCO#	45K4613
194	MHW-E	1994	Nikko	2802	GIBCO#	45K4613
198	MHW-E	1994	Nikko	2802	GIBCO#	45K4613
140	MHW-F	1994	Nikko	2802	GIBCO#	45K4613
157	MHW-F	1994	Nikko	2802	GIBCO#	45K4613
123	MHW-F	1994	Nikko	2802	GIBCO#	45K4613
174	MHW-Achilles	1994	Nikko	2802	GIBCO#	45K4613
189	MHW-Achilles	1994	Nikko	2802	GIBCO#	45K4613
176	MHW-Achilles	1994	Nikko	2802	GIBCO#	45K4613
102.2	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
87.2	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.1	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
90.5	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
90.5	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
103.1	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
101.7	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
90.6	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
95.1	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
96.1	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.4	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
92.4	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
96.2	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
90.3	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
98.8	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
101.7	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
108	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
104.2	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
92.7	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
100.2	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
97.2	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
103.5	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
113.7	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
107.2	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
93.5	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
85.9	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.8	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.2	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
92.1	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
96.9	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.9	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
96	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
86.3	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
93.4	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
95.2	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.8	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
95.5	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
93.3	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
96	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
94	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
90.7	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
89.9	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
90.8	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
94.4	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
96.6	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
90	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
92	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.5	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.5	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
90.7	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
92.2	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
89.1	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
93	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
98.7	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
93.6	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
96.5	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
100.6	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.3	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
93.8	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
89.1	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
96.6	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
92.8	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
94.4	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.4	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML

IC50 (μg/mL)	Lab.	Year	Manufacturer	Lot.	Manufacturer of serum	Lot of serum
90.8	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
89.1	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.8	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
93.2	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
95.1	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
92.5	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
93.1	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
92.4	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
96.5	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
89.6	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
89.4	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
86	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
94.8	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
96.7	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
89.7	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
88.1	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.5	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.3	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
86	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.4	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.5	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
89.1	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
90.6	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
89	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
96.4	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
93	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
90.4	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
92.8	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
95.3	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
87.1	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
90.4	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
113.6	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
92.4	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.1	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
94.3	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
88.2	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
93.9	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
92.9	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.7	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.1	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
92.5	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
90.1	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
89.7	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
94.3	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
92.9	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
92.7	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
93.6	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
109.2	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.3	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
92.2	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
93.5	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
87.2	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
101.6	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
89.7	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.5	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
93.8	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.8	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
93.7	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
93.5	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
92.1	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
95.2	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
92.6	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
91.9	Shiseido	2009	Wako	TCG8149	JRH	12603C-500ML
90.7	Shiseido	2010	Wako	TCG8149	JRH	12603C-500ML
95.1	Shiseido	2010	Wako	TCG8149	JRH	12603C-500ML

JRH:JRH Bioscience
 Nichirei:Nichirei Biosciences Inc.
 Wako: Wako Pure Chemical Industries, Ltd.
 #:Retest

§:Calf serum was obtained from GIBCO Laboratories (NY, USA)

Difference by manufacturer and lot of SDS

Manufacturer of SDS	Lot of SDS	n	IC50 (µg/mL)	
			Average	SD
Wako	SDF8155	171	88.3	4.8
Wako	TCG8149	134	93.5	4.9
Nikko Chemicals	2802	18	165.9	30.0

Other difference of test condition
-
Lab., Manufacturer of serum
Lab., Type of serum (Calf serum)

Difference by manufacturer and lot of serum

Manufacturer of serum	Lot of serum	n	IC50 (µg/mL)	
			Average	SD
GIBCO	909463	12	82.0	4.5
GIBCO	1073767	135	89.2	4.4
Nichirei	9E0887	24	85.8	4.2
JRH	2603C-500ML	134	93.5	4.9
GIBCO ³ (Calf serum)	45K4613	18	165.9	30.0

Other difference of test condition
-
Lab.
Lab.
Lab., Lot of SDS
Lab., Manufacturer of SDS

Appendix 8.10 Effect of solvents in the validation study

The average \pm standard deviation of the O.D. showing viable cells after application of each solvent was analyzed from the viewpoint of effect of solvents. The negative control was 0.64 ± 0.08 in the Medium (n = 52) and 0.66 ± 0.08 in medium containing DMSO (n = 28), as calculated from Phase III data obtained at Lab A, and 0.97 ± 0.09 in the Medium (n = 76) and 0.93 ± 0.10 in medium containing ethanol (n = 4), as calculated from Phase III data obtained at Lab B. Neither Lab A nor Lab C used ethanol as a solvent, nor did Lab B use DMSO as solvent. No effect of used solvents was confirmed from this validation data.

(1)Effect of DMSO

No.	IC50 ($\mu\text{g/mL}$)	IC50 ($\mu\text{g/mL}$)	Solvent (M:Medium, D:Medium containing DMSO)	OD	OD
P3-003	212.8	259.2	M	0.636	0.649
P3-005	>5000	>5000	M	0.708	0.669
P3-010	1323.3	1653.3	M	0.691	0.583
P3-012	1460.9	1541.2	M	0.648	0.711
P3-019	155.8	202.5	D	0.681	0.652
P3-020	1347.4	1588.5	D	0.602	0.543
P3-022	<39.1	42.4	M	0.879	0.69
P3-024	151.8	182.9	M	0.732	0.534
P3-027	484.9	869.1	M	0.724	0.651
P3-028	<39.1	<39.1	M	0.657	0.608
P3-029	42.2	46	D	0.698	0.591
P3-033	>5000	>5000	M	0.489	0.58
P3-042	<39.1	<39.1	D	0.834	0.594
P3-045	117.7	128.7	D	0.814	0.563
P3-073	444.1	470.6	M	0.78	0.654
P3-074	52.1	47.5	M	0.696	0.556
P3-075	<39.1	<39.1	D	0.711	0.631
P3-076	946.3	761.9	M	0.644	0.631
P3-077	>5000	>5000	M	0.589	0.62
P3-078	>5000	>5000	M	0.736	0.576

P3-079	>5000	>5000	M	0.688	0.524
P3-080	1082.2	1666.5	D	0.606	0.536
P3-081	84.6	352	D	0.559	0.551
P3-082	777.3	857.3	D	0.705	0.653
P3-083	>5000	>5000	M	0.628	0.537
P3-084	4903.1	>5000	M	0.688	0.596
P3-085	2243.5	3624.5	M	0.789	0.599
P3-086	2243.5	3624.5	M	0.606	0.539
P3-087	>5000	3648	M	0.684	0.688
P3-088	1941.1	2253.7	M	0.699	0.534
P3-089	>5000	>5000	M	0.541	0.561
P3-090	<39.1	<39.1	D	0.704	0.628
P3-093	682.6	866.2	M	0.664	0.539
P3-094	1429.5	1504.2	M	0.784	0.646
P3-095	1864.4	1696.9	D	0.648	0.615
P3-096	94.3	67	D	0.837	0.703
P3-097	132.4	274.5	D	0.748	0.657
P3-098	190	168.8	D	0.674	0.724
P3-099	1133.6	1574.3	M	0.818	0.633
P3-100	2043.9	2606.8	M	0.64	0.545

	N	Average	SD
Medium	52	0.64	0.08
DMSO	28	0.66	0.08

(2)Effect of Ethanol

No.	IC50 (µg/mL)	IC50 (µg/mL)	Solvent (M:Medium, E:Medium containing Ethanol)	OD	OD
P3-001	119.6	122.6	M	0.981	1.036
P3-003	695.2	672.8	E	0.862	0.97
P3-005	>5000	>5000	M	1.02	1.149
P3-008	17.7	22.8	M	1.062	0.91
P3-010	626.8	535.2	M	0.976	0.99

P3-012	814.2	768.8	M	1.029	1.094
P3-019	265.5	187.4	M	1.072	0.977
P3-020	2923.4	2017.9	M	0.981	0.959
P3-024	71.7	63.1	M	0.853	0.966
P3-028	6.9	11.7	M	0.821	0.842
P3-029	<39.1	<39.1	M	0.992	0.853
P3-033	4864.9	4126.6	M	1.095	1.05
P3-043	163.3	191.9	M	1.006	0.919
P3-046	783.5	346.3	M	0.865	0.913
P3-047	1599.2	1570.6	M	0.913	0.848
P3-048	2203.1	2105	M	1.021	1.037
P3-049	772.6	414.8	M	0.847	0.957
P3-050	>5000	>5000	M	0.961	1.151
P3-051	128.7	312.5	M	1.011	0.93
P3-052	92.1	98.3	M	0.954	0.9
P3-053	720.4	213.4	M	0.858	0.751
P3-054	195.5	169.9	M	0.961	0.951
P3-055	17.3	20.6	M	1.065	0.946
P3-056	>5000	>5000	M	1.102	1.074
P3-057	>5000	>5000	M	0.989	0.888
P3-058	11.3	13.9	M	1.098	0.972
P3-059	>5000	>5000	M	1.037	0.967
P3-060	1343.6	1473.8	M	0.968	0.973
P3-061	620.5	604.4	M	1.027	1.136
P3-062	1729.4	1824.4	M	0.805	1.036
P3-063	>2500	>2500	M	1.028	0.857
P3-064	1619	1403.1	M	0.87	1.081
P3-065	1604.1	1429.4	M	0.805	0.891
P3-066	>315	>315	M	0.899	0.969
P3-067	875.3	807.7	M	0.97	1.038
P3-068	1584.6	1468.4	M	0.883	1.024
P3-069	1276	1587.5	M	0.935	0.812
P3-070	3.6	14	E	1.049	0.838
P3-071	97.5	70.7	M	0.923	0.971
P3-072	57.2	60.1	M	1.067	0.824

	N	Average	SD
Medium	76	0.97	0.09
Ethanol	4	0.93	0.10

Appendix 8.11 Analysis of predictive capacity by the data from this validation study and the additional data from Shiseido

The predictive capacity of SIRC-CVS:TEA test was analyzed by the data from this validation study and the additional data from Shiseido. Shiseido's data were taken from the report used in the peer review by JaCVAM eye irritation test evaluating committee in 2009-2011, and Their data sheets was checked during the peer review. Table 1 shows the data of 33 substances (Purity \geq 80%) for the analysis, except for the overlapped 21 substances of this validation and Shiseido. The predictive capacity by 33 substances was an accuracy of 63.6% (21/33), a sensitivity of 76.5% (13/17), and a specificity of 50.0% (8/16), as shown in table 2. Also, when excluding chemicals such as alcohols, esters, ethers, ketones, heterocyclic compounds, and carboxylic acid compounds with a molecular weight of less than 180, the predictive capacity was an accuracy of 63.6% (14/22), a sensitivity of 100% (11/11), and a specificity of 27.3% (3/11), as shown in table 3.

The predictive capacity by the 57 data from this validation study and the additional 22 data from Shiseido was an accuracy of 64.6% (51/79), a sensitivity of 94.6% (35/37), and a specificity of 38.1% (16/42). Also, false negative rate was 5.4% (2/37) and false positive rate was 61.9% (26/42), as shown in table 4 and 5.

Table 1 Additional data for analysis of predictive capacity of SIRC-CVS:TEA test

	Substance	CAS	in vitro	in vivo ¹⁾	MW	Purity ²⁾
1	Butylene glycol	107-88-0	N	N	90.1	min.98.0%
2	Propylene carbonate	108-32-7	N	N	102.1	min.97.0%
3	2,4-Pentanediol	625-69-4	N	N	104.2	98%
4	Resorcinol	108-46-3	P	P	110.1	99.0+%
5	Butoxyethanol	111-76-2	N	P	118.2	min.99.0%
6	Hexylene glycol	107-41-5	N	P	118.2	99.0+%
7	Phenethyl alcohol	60-12-8	P	P	122.2	98.0+%
8	Methoxyisopropyl acetate	108-65-6	N	P	132.2	99%
9	6-Methyl purine	2004-03-7	P	P	134.1	\geq 99%
10	Phenoxyethanol	122-99-6	N	P	138.2	min.99.0%
11	Di-iso-butyl ketone	108-83-8	N	N	142.2	\geq 99%

12	Triethylene glycol	112-27-6	N	N	150.2	95.0+%
13	Chloroxylonol	88-04-0	P	P	156.6	98.0+%
14	2,4-Difluoronitrobenzene	446-35-5	P	N	159.1	99%
15	iso-Octyl acrylate	29590-42-9	P	N	184.3	>90%
16	Sodium dehydroacetate	4418-26-2	P	N	190.1	95.0+%
17	Triisopropanolamine	122-20-3	P	P	191.3	95.0+%
18	2-Bromo-2-Nitropropane-1,3-Diol	52-51-7	P	P	200.0	98+%
19	Benzophenone-1	131-56-6	P	P	214.2	98.0+%
20	Triacetin	102-76-1	P	N	218.2	98.0+%
21	Chlorophene	120-32-1	P	P	218.7	≥97.0%
22	Sodium naphthalenesulfonate	532-02-5	P	P	230.2	min.90.0%
23	Diisopropyl adipate	6938-94-9	P	N	230.3	98.0+%
24	tetra-Aminopyrimidine sulfate	5392-28-9	P	N	238.2	97%
25	Cetyl alcohol	36653-82-4	P	N	242.4	95.0+%
26	Benzophenone-2	131-55-5	P	P	246.2	95.0+%
27	Oleyl alcohol	143-28-2	P	N	268.5	99%
28	Isopropyl Palmitate	142-91-6	N	N	298.5	95.0+%
29	Cetrimonium chloride	112-02-7	P	P	320.0	95.0+%
30	Diethylhexyl adipate	103-23-1	N	N	370.6	99.0+%
31	Squalane	111-01-3	N	N	422.8	98.0+%
32	Stearalkonium chloride	122-19-0	P	P	424.2	85.0+%
33	Diocetyl sodium sulfosuccinate	577-11-7	P	P	488.5	96%

1) In vivo data is taken from the previous paper.

2) Purity is that of the substances used at in vitro test.

Table 2 Predictive capacity of 33 substances

N=33		In vitro	
		Positive	Negative
In vivo	Positive	13	4
	Negative	8	8

Table 3 Predictive capacity of 22 substances except for alcohols, esters, ethers, ketones, heterocyclic compounds, and carboxylic acid compounds with a molecular weight of less than 180

N=22		In vitro	
		Positive	Negative
In vivo	Positive	11	0
	Negative	8	3

Table 4 The predictive capacity by the data from this validation study and the additional data from Shiseido

N=79		In vitro	
		Positive	Negative
In vivo	Positive [#]	35	2
	Negative	26	16

Table 5 The predictive capacity of test substances (Purity \geq 80%) except for alcohol, ester, ether, ketone, heterocyclic compound and carboxylic acid of molecular weight <180

Regulatory System	Analysis in applicability domain
Accuracy	64.6% (51/79)
Sensitivity	94.6% (35/37)
Specificity	38.1% (16/42)
False Negative Rate	5.4% (2/37)
False Positive Rate	61.9% (26/42)