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# Supporting Information

# Disulfide Symmetric Dimers as Stable Pre-Hapten Forms for Bioconjugation. A Strategy to Prepare Immunoreagents for the Detection of Sulfophenylcarboxylates (SPC) Residues in Environmental Samples

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**RESULTS**

**Antibody Detectability**. Screening of the ability of the antiserums to recognize the **$SPC_{MIX}$** (a mixture of the six short chain SPCs **$2C_3$, $2C_4$, $2C_5$, $3C_4$, $3C_5$** and **$3C_6$-SPC)** was determined through competitive assays by evaluating 225 antisera/coating antigen combinations regarding recognition of two different SPCs concentrations: a higher one ($I_1$=150 μM) and a lower one ($I_2$= 200 nM). Table A summarizes the result of this screening in terms of the decrease in the absorbance produced by these SPC solutions in comparison to the signal at zero SPC concentration. It can be observed that the antisera raised against **type A** and **type AB** immunogens recognized the **SPCs** much more when **type B** antigens were used as competitors. In contrast, the antisera raised against **type B** haptens recognized very well the **SPCs** with both types of competitors, although a higher number of usable combinations were found when the haptenized coating antigens used were also of **type B**. At this stage, it could not be stated whether if the homologous (a single antigenic determinant, the carboxylic or the sulfonic group) or *pseudo*-heterologous (both antigenic determinants) antisera recognized better the SPCs. Further experiments were performed choosing, for each type of antisera (**A**, **B** and **AB**), those combinations showing an inhibition of the absorbance greater than 50% for $I_1$ and at least of 15% for $I_2$ (combinations giving VH and H inhibition degrees, see table A). About 150 As/Antigen combinations were then evaluated after selecting the most appropriate immunoreagent concentrations through two-dimensional checkerboard titration experiments (see Table B1, B2 and B3). The complete results of the competitive assays for these combinations are summarized in the Table C.

**Evaluation of Immunoassay Perfomance.** Assays **As112/$2C_5$-OVA**, **As115/$2C_5$-S-OVA** and **As119/$2C_3$-BSA,** were further evaluated to determine detectability and specificity of the antisera raised by determining first immunoassay performance under different conditions. The effect of several physico-chemical parameters on the immunoassay features was studied for the three combinations (see figure A). Thus, no significant effects were observed over the maximum signal and the $IC_{50}$ values of the assays when *the concentration of Tween 20* varied from 0.2 to 0.01%. Increasing the *conductivity* (from 4.3 to 65.3 mS $cm^{-1}$) produced a general decrease in the maximum signal but the detectability remained almost constant. Only, a slight decrease of the $IC_{50}$ value was produced in media with a low ionic strength. Although short incubation times (15 min) increased slightly the detectability, the *length of the competition step* was set to 30 min since a decrease in the maximum absorbance was also observed. *Preincubatig* the antibody with the analyte for 1h produced a slight improvement of the detectability, however the

reproducibility was worse. All assays did work very well in assay media with pH values within 4.5 and 9.5 units. More extreme pHs led to an important decrease in the maximum signal, especially at acidic pH values. Although a slight improvement in the sensitivity could be observed when the pH ranged between 4.5 and 5.5, changing the buffer system from PBS to citrate, produced an important decrease in the maximum absorbance.

## EXPERIMENTAL SECTION

### A) Organic Chemistry

**Phenylcarboxylate esters (1'-7'):**

***2-Phenylpropionic butyl ester (1').*** (206 mg, 76% yield). $^{1}$H NMR (300 MHz, $CDCl_3$): d(ppm) 0.87 (t, J=7Hz, 3H, -$CH_3$), 1.28 (tq, J=8Hz, J=7.5Hz, 2H, -$CH_2$-), 1.50 (d, J=7Hz, 3H, -$CH_3$), 1.56 (tt, J=7Hz, J=6.5Hz, 2H, -$CH_2$-), 3.71 (q, J=7Hz, 1H, -CH-), 4.06 (t, J?=6.5Hz, 2H, $OCH_2$-), 7.26-7.32 (m, 5H, $H_{Ar}$). $^{13}$C NMR (75 MHz, $CDCl_3$): d(ppm) 13.6 (-$CH_3$), 18.5 (C-3), 19.0 (-$CH_2$-), 30.6 (-$CH_2$), 45.6 (C-2), 64.6 ($OCH_2$-), 127.0 (C-4'), 127.5 (C-3'), 128.5 (C-2'), 140.7 (C-1'), 174.0 (C-1). IR, ν, (KBr, $cm^{-1}$): 2960-2875 (C-H st), 1733 (C=O st), 1170 (C-O st as).

***2-Phenylbutyric butyl ester (2').*** (2 g, 75% yield). $^{1}$H NMR (300 MHz, $CDCl_3$): d(ppm) 0.83 (t, J=7Hz, 3H), 0.86 (t, J=7Hz, 3H), 1.25 (tq, J=7.5Hz, J=7.5Hz, 2H), 1.52 (tt, J=7Hz, J=7Hz, 2H), 1.75 (tq, J=7.5HZ, J=7Hz, 1H), 2.06 (tq, J=7.5Hz, J=7Hz, 1H), 3.40 (t, J=8Hz, 1H), 3.99 (dt, J=11Hz, J=6.5Hz, 1H), 4.05 (dt, J=11Hz, J=6.5Hz, 1H), 7.26-7.32 (m, 5H). $^{13}$C NMR (75 MHz, $CDCl_3$): d(ppm) 12.2, 13.6, 19.0, 26.7, 30.6, 53.6, 64.5, 127.1, 127.9, 128.5, 139.2, 174.1. IR, ν, (KBr, $cm^{-1}$): 2964-2875 (C-H st), 1733 (C=O st), 1166 (C-O st as).

***2-Phenylpentanoic butyl ester (3').*** (2.6 g, 71% yield). $^{1}$H NMR (300 MHz, $CDCl_3$): d(ppm) 0.88 (t, J=7.5Hz, 3H), 0.91 (t, J=7Hz, 3H), 1.28 (m, 4H), 1.56 (tt, J=7Hz, J=7Hz, 2H), 1.75 (tq, J=7Hz, J=6.5Hz, 1H), 2.05 (tq, J=7Hz, J=6.5Hz, 1H), 3.54 (t, J=8Hz, 1H), 4.03 (dt, J=11Hz, J=6.5Hz, 1H), 4.08 (dt, J=11Hz, J=6.5Hz, 1H), 7.26-7.32 (m, 5H). $^{13}$C NMR (75 MHz, $CDCl_3$): d(ppm) 13.6, 13.8, 19.0, 20.8, 30.6, 35.6, 51.6, 64.5, 127.0, 127.9, 128.5, 139.1, 174.2.

***3-Phenylbutyric butyl ester (4').*** (2.33 g, 87% yield). $^{1}$H NMR (300 MHz, $CDCl_3$): d(ppm) 0.89 (t, J=7.5Hz, 3H), 1.29 (tq, J=7Hz, J=7Hz, 2H), 1.30 (d, J=7Hz, 3H), 1.53 (tt, J=7.5Hz, J=7Hz, 2H), 2.54 (dd, J=8Hz, J=8Hz, 1H), 2.62 (dd, J=8Hz, J=8Hz, 1H), 3.27 (tq, J=7Hz, J=7Hz, 1H), 4.02 (t, J=6.5Hz, 2H), 7.23-7.31 (m, 5H). $^{13}$C NMR (75 MHz, $CDCl_3$): d(ppm) 13.7, 19.0, 21.9,

30.6, 36.5, 43.0, 64.2, 126.4, 126.7, 128.5, 145.7, 172.5. IR, ν, (KBr, $cm^{-1}$): 2961-2873 (C-H st), 1735 (C=O st), 1166 (C-O st as).

***3-Phenylpentanoic butyl ester (5').*** (5.45 g, 82% yield). $^{1}H$ NMR (300 MHz, $CDCl_3$): d(ppm) 0.86 (t, J=7.5Hz, 3H), 0.94 (t, J=7Hz, 3H), 1.32 (tq, J=7Hz, J=7Hz, 2H), 1.55 (m, 2H), 1.70 (m, 2H), 2.62 (dd, J=15Hz, J=8Hz, 1H), 2.72 (dd, J=15Hz, J=7Hz, 1H), 3.06 (tt, J=8.5Hz, J=8.5Hz, 1H), 4.04 (t, J=6.5Hz, 2H), 7.22-7.35 (m, 5H).

***3-Phenylhexanoic methyl ester (6').*** (425 mg, 80% yield). $^{1}H$ NMR (300 MHz, $CDCl_3$): d(ppm) 0.85 (t, J=7.5Hz, 3H), 1.17 (m, 2H), 1.62 (m, 2H), 2.60 (dd, J=16Hz, J=8.5Hz, 1H), 2.71 (dd, J=16.5Hz, J=6Hz, 1H), 3.08 (tt, J=8Hz, J=8Hz, 1H), 3.59 (s, 3H), 7.16-7.31 (m, 5H).

***5-Phenylpentanoic methyl ester (7').*** (5.04 g, 93% yield). $^{1}H$ NMR (300 MHz, $CDCl_3$): d(ppm) 1.66 (m, 4H), 2.34 (t, J=7Hz, 2H), 2.63 (t, J=7Hz, 2H) 3.66 (s, 3H), 7.16-7.28 (m, 5H). $^{13}C$ NMR (75 MHz, $CDCl_3$): d(ppm) 24.6, 30.9, 33.9, 35.5, 51.5, 125.7, 128.3, 128.4, 142.1, 174.1.

**Chlorosulfonyl derivatives (1a-7a and 1b-3b):**

***2-(4-Chlorosulfonyl-phenyl) propionic butyl ester. (1a).*** (600 mg, 20% yield). $^{1}H$ NMR (300 MHz, $CDCl_3$): d(ppm) 0.88 (t, J=7Hz, 3H, $-CH_3$), 1.29 (tq, J=7.5Hz, J=7.5Hz, 2H, $-CH_2-$), 1.55 (d, J=7Hz, 3H, $CH_3-$), 1.57 (tt, J=6.5Hz, J=6.5Hz, 2H, $-CH_2-$), 3.84 (q, J=7Hz, 1H, -CH-), 4.10 (t, J=6.5Hz, 2H, $OCH_2-$), 7.56 (d, J=8.5Hz, $2H_{Ar}$, *ortho*), 8.00 (d, J=8.5Hz, $2H_{Ar}$, *meta)*. $^{13}C$ NMR (75 MHz, $CDCl_3$): d(ppm) 13.6 ($-CH_3$), 18.5 (C-3), 19.0 ($-CH_2-$), 30.6 ($-CH_2$), 45.6 (C-2), 65.2 ($OCH_2-$), 127.3 (C-3'), 128.9 (C-2'), 143.0 (C-4'), 148.6 (C-1'), 173.0 (C-1). IR, ν, (KBr, $cm^{-1}$): 2962-2875 (C-H st), 1733 (C=O st), 1593 (ArC-C), 1379 ($-SO_2Cl$ st as), 1176 (C-O st si).

***2-(4-Chlorosulfonyl-phenyl) butyric butyl ester. (2a).*** (460 mg, 32% yield). $^{1}H$ NMR (300 MHz, $CDCl_3$): d(ppm) 0.89 (t, J=7.5Hz, 3H), 0.92 (t, J=7.5Hz, 3H), 1.30 (tq, J=7.5Hz, J=7.5Hz, 2H), 1.58 (tt, J=7Hz, J=7Hz, 2H), 1.82 (ddq, J=15Hz, J=7.5HZ, J=7Hz, 1H), 2.15 (ddq, J=15Hz, J=7.5Hz, J=7Hz, 1H), 3.58 (t, J=8Hz, 1H), 4.06 (dt, J=11Hz, J=6.5Hz, 1H), 4.12 (dt, J=11Hz, J=6.5Hz, 1H), 7.57 (d, J=8.5Hz, 2H), 7.99 (d, J=8.5Hz, 2H*)*. $^{13}C$ NMR (75 MHz, $CDCl_3$): d(ppm) 12.1, 13.6, 19.0, 26.8, 30.5, 53.5, 65.1, 127.2, 129.4, 143.0, 147.3, 172.6. IR, ν, (KBr, $cm^{-1}$): 2963-2875 (C-H st), 1731 (C=O st), 1593 (ArC-C), 1378 ($-SO_2Cl$ st as), 1176 (C-O st si).

***2-(4-Chlorosulfonyl-phenyl) pentanoic butyl ester. (3a).*** (940 mg, 37% yield). $^{1}$H NMR (300 MHz, $CDCl_3$): d(ppm) 0.88 (t, J=8Hz, 3H), 0.93 (t, J=7Hz, 3H), 1.29 (m, 4H), 1.57 (tt, J=7Hz, J=7Hz, 2H), 1.77 (ddt, J=15.5Hz, J=7.5Hz, J=6Hz, 1H), 2.09 (ddt, J=15.5Hz, J=7.5Hz, J=6Hz, 1H), 3.68 (t, J=8Hz, 1H), 4.05 (dt, J=11Hz, J=6.5Hz, 1H), 4.11 (dt, J=11Hz, J=6.5Hz, 1H), 7.57 (d, J=8.5Hz, 2H), 7.98 (d, J=8.5Hz, 2H). $^{13}$C NMR (75 MHz, $CDCl_3$): d(ppm) 13.6, 13.7, 19.0, 20.7, 30.5, 35.6, 51.6, 65.1, 127.2, 129.3, 143.0, 147.5, 172.7. IR, ν, (KBr, $cm^{-1}$): 2960-2873 (C-H st), 1733 (C=O st), 1593 (ArC-C), 1378 (-$SO_2$Cl st as), 1176 (C-O st si).

***3-(4-Chlorosulfonyl-phenyl) butyric butyl ester. (4a).*** (1.08 g, 50%yield). $^{1}$H NMR (300 MHz, $CDCl_3$): d(ppm) 0.88 (t, J=7.5Hz, 3H), 1.27 (tq, J=8Hz, J=8Hz, 2H), 1.34 (d, J=7Hz, 3H), 1.52 (tt, J=7 Hz, J=6.5Hz, 2H), 2.63 (d, J=7.5, 2H), 3.41 (tq, J=7Hz, J=7Hz, 1H), 4.01 (t, J=6.5Hz, 2H), 7.47 (d, J=8.5Hz, 2H), 7.97 (d, J=8.5Hz, 2H). $^{13}$C NMR (75 MHz, $CDCl_3$): d(ppm) 13.6, 19.0, 21.7, 30.5, 36.6, 42.1, 64.5, 127.3, 128.2, 142.4, 154.1, 171.6. IR, ν, (KBr, $cm^{-1}$): 2962-2875 (C-H st), 1733 (C=O st), 1593 (ArC-C), 1376 (-$SO_2$Cl st as), 1174 (C-O st as).

***3-(4-Chlorosulfonyl-phenyl) pentanoic butyl ester. (5a).*** (880 mg, 41% yield). $^{1}$H NMR (300 MHz, $CDCl_3$): d(ppm) 0.81 (t, J=7.5Hz, 3H), 0.86 (t, J=7Hz, 3H), 1.24 (tq, J=7.5Hz, J=7.5Hz, 2H), 1.47 (tt, J=6.5Hz, J=6.5Hz, 2H), 1.64 (ddq, J=13.5Hz, J=7.5Hz, J=6.5Hz, 1H), 1.76 (ddq, J=13.5Hz, J=7.5Hz, J=6.5Hz, 1H), 2.59 (dd, J=15.5Hz, J=9Hz, 1H), 2.71 (dd, J=15.5Hz, J=6.5Hz, 1H), 3.15 (tt, J=9Hz, J=6Hz, 1H), 3.98 (t, J=6.5Hz, 2H), 7.43 (d, J=8.5Hz, 2H), 7.96 (d, J=8.5Hz, 2H). $^{13}$C NMR (75 MHz, $CDCl_3$): d(ppm) 11.8, 13.6, 19.0, 29.0, 30.5, 40.7, 44.0, 64.5, 127.2, 128.9, 142.4, 152.7, 171.7. IR, ν, (KBr, $cm^{-1}$): 2962-2875 (C-H st), 1731 (C=O st), 1592 (ArC-C), 1376 (-$SO_2$Cl st as), 1174 (C-O st as).

***3-(4-Chlorosulfonyl-phenyl) hexanoic methyl ester. (6a).*** (257 mg, 43% yield). $^{1}$H NMR (300 MHz, $CDCl_3$): d(ppm) 0.88 (t, J=7. Hz, 3H), 1.18 (tq, J=7Hz, J=7Hz, 2H), 1.64 (m, 2H), 2.60 (dd, J=16Hz, J=8.5Hz, 1H), 2.71 (dd, J=16.5Hz, J=6.5Hz, 1H), 3.26 (tt, J=8.5Hz, J=6Hz, 1H), 3.59 (s, 3H), 7.44 (d, J=8.5Hz, 2H), 7.97 (d, J=8.5Hz, 2H). $^{13}$C NMR (75 MHz, $CDCl_3$): d(ppm) 13.8, 20.4, 38.1, 40.7, 41.9, 51.7, 127.2, 128.8, 142.4, 152.9, 172.0. IR, ν, (KBr, $cm^{-1}$): 2958-2873 (C-H st), 1737 (C=O st), 1593 (ArC-C), 1377 (-$SO_2$Cl st as), 1174 (C-O st as).

***5-(4-Chlorosulfonyl-phenyl) pentanoic methyl ester. (7a).*** (3 g, 40% yield). $^{1}$H NMR (300 MHz, $CDCl_3$): d(ppm) 1.70 (m, 4H), 2.36 (t, J=7Hz, 2H), 2.76 (t, J=7Hz, 2H) 3.68 (s, 3H), 7.42 (d, J=8.5Hz, 2H), 7.95 (d, J=8.5Hz, 2H). $^{13}$C NMR (75 MHz, $CDCl_3$): d(ppm) 24.4, 30.2, 33.7,

35.7, 51.6, 127.2, 129.6, 142.0, 150.7,173.7. IR, ν, (KBr, $cm^{-1}$): 2952-2935 (C-H st), 1733 (C=O st), 1589 (ArC-C), 1377 (-$SO_2$Cl st as), 1323 (C-O st as), 1174 (C-O st as).

***2-(3-Chlorosulfonyl-phenyl) propionic butyl ester. (1b).*** (360 mg, 12% yield). $^1$H NMR (300 MHz, $CDCl_3$): d(ppm) 0.87 (t, J=7.5Hz, 3H), 1.29 (tq, J=7.5Hz, J=7.5Hz, 2H), 1.56 (tt, J=6.5Hz, J=6.5Hz, 3H), 1.57 (d, J=7Hz, 2H), 3.84 (q, J=7Hz, 1H), 4.09 (t, J=6.5Hz, 2H), 7.59 (dd, J=8Hz, J=8Hz, 1H), 7.70 (d, J=8Hz, 1H), 7.95 (d, J=8Hz, 1H), 7.98 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$): d(ppm) 13.6, 18.3, 19.0, 30.4, 45.3, 65.2, 125.7, 126.1, 129.9, 134.5, 142.9, 144.5, 173.2. IR, ν, (KBr, $cm^{-1}$): 2962-2875 (C-H st), 1733 (C=O st), 1593 (ArC-C), 1379 (-$SO_2$Cl st as), 1174 (C-O st si).

***2-(3-Chlorosulfonyl-phenyl) butyric butyl ester. (2b).*** (86 mg, 6% yield). $^1$H NMR (300 MHz, $CDCl_3$): d(ppm) 0.87 (t, J=7.5Hz, 3H), 0.92 (t, J=7.5Hz, 3H), 1.29 (tq, J=7.5Hz, J=7.5Hz, 2H), 1.57 (tt, J=7Hz, J=7Hz, 2H), 1.83 (ddq, J=17.5Hz, J=7.5HZ, J=7Hz, 1H), 2.15 (ddq, J=15Hz, J=7.5Hz, J=7Hz, 1H), 3.58 (t, J=8Hz, 1H), 4.07 (dt, J=11Hz, J=6.5Hz, 1H), 4.12 (dt, J=11Hz, J=6.5Hz, 1H), 7.58 (t, J=8.5Hz, 2H), 7.71 (d, J=8Hz, 1H), 7.94 (d, J=8Hz, 1H), 7.98 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$): d(ppm) 12.0, 13.6, 19.0, 26.7, 30.5, 53.2, 65.0, 125.7, 126.5, 129.8, 134.8, 141.6, 144.5, 172.6. IR, ν, (KBr, $cm^{-1}$): 2964-2875 (C-H st), 1731 (C=O st), 1593 (ArC-C), 1378 (-$SO_2$Cl st as), 1174 (C-O st si).

***2-(3-Chlorosulfonyl-phenyl) pentanoic butyl ester. (3b).*** (254 mg, 10% yield). $^1$H NMR (300 MHz, $CDCl_3$): d(ppm) 0.88 (t, J=8Hz, 3H), 0.93 (t, J=7Hz, 3H), 1.30 (m, 4H), 1.58 (tt, J=7Hz, J=7Hz, 2H), 1.77 (ddt, J=15.5Hz, J=7.5Hz, J=6Hz, 1H), 2.10 (ddt, J=15.5Hz, J=7.5Hz, J=6Hz, 1H), 3.68 (t, J=8Hz, 1H), 4.07 (dt, J=11Hz, J=6.5Hz, 1H), 4.12 (dt, J=11Hz, J=6.5Hz, 1H), 7.58 (t, J=8Hz, 1H), 7.72 (d, J=8Hz, 1H), 7.94 (d, J=8Hz, 1H), 7.99 (s, 1H). $^{13}$C NMR (75 MHz, $CDCl_3$): d(ppm) 13.6, 13.7, 19.0, 20.7, 30.5, 35.6, 51.3, 65.1, 125.7, 126.5, 129.8, 134.8, 141.8, 144.5, 172.8. IR, ν, (KBr, $cm^{-1}$): 2962-2875 (C-H st), 1733 (C=O st), 1593 (ArC-C), 1380 (-$SO_2$Cl st as), 1174 (C-O st si).

### B) Immunochemistry

**Buffers**. Phosphate-buffered saline (PBS) is 0.01 M phosphate buffer with 0.8% saline solution, pH 7.5. PBST is PBS with 0.05% Tween 20. PBST-BSA is PBST with 1% BSA. Borate buffer is 0.2 M boric acid-sodium borate, pH 8.7. Coating buffer is 0.05 M carbonate/bicarbonate buffer, pH 9.6. Citrate buffer is a 0.04 M solution of sodium citrate, pH 5.5. The substrate

solution contains 0.01% TMB (3,3',5,5'-tetramethylbenzidine) and 0.004% $H_2O_2$ in citrate buffer.

**Preparation of the Immunoreagents using Haptens Type B.** Haptens **$2C_3$-SPC, $2C_4$-SPC, $2C_5$-SPC, $3C_4$-SPC, $3C_5$-SPC, $3C_6$-SPC, $5C_5$-SPC,** and **$9C_9$-SPCs** were coupled to BSA by using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC). Briefly, the hapten (10 μmol in 100 μL of PBS) was added to a solution of BSA (10mg, 5 μmol lysines in 0.9 mL of PBS buffer) followed by a solution of EDC (50 μmol in 100 μL of PBS). The solution was left under stirring at room temperature for 4h. All the conjugates were extensively dialyzed as described before, lyophilized and stored frozen at -40ºC. Working aliquots (1 mg $mL^{-1}$) in 10 mM PBS were stored 4°C. The degree of conjugation was assessed by MALDI-TOF-MS.

**Hapten density analysis.** Hapten densities were calculated by MALDI-TOF-MS (matrix-assisted laser desorption ionization time-of-flight mass spectrometry) by determining the molecular weight (MW) of the native protein and that of the corresponding **Hapten-Protein** conjugates for **type B** haptens. For haptens **type A** were recorded also the mass spectra of the corresponding **Hapten-MP-(or -$CH_2$)-protein** and **MP-Protein or BrA-Protein** conjugates. MALDI spectra were obtained by mixing 2 μL of the matrix (*trans*-3,5-dimethoxy-4-hydroxycinnamic acid, 10 mg $mL^{-1}$ in $CH_3CN/H_2O$ 70:30, 0.1% TFA) with 2 μL of a solution of the conjugates or proteins (5 mg $mL^{-1}$ in MilliQ water). For the **type A** conjugates, the efficiency of the step 2 and of the step 3 was evaluated according to the cross-linker:protein (*CL:P*) and hapten:protein (*H:P*) molar ratios, which were calculated according to the following equations:

$$CL:P = \{MW(MP\text{-}Prot \text{ or } BrA\text{-}Prot) - MW(Prot)\} / MW(MP \text{ o } BrA)\}$$

$$(H:P)^{M}_{A} = \{MW(Hapten\text{-}MP\text{-}Prot) - MW(MP\text{-}Prot)\} / MW(Hapten)\} \text{ or}$$

$$(H:P)^{B}_{A} = \{MW(Hapten\text{-}CH_2\text{-}Prot) - MW(BrA\text{-}Prot) - MW(Br)\} / MW(Hapten)\}$$

Hapten:protein molar ratios for **type B** immunoreagents was calculated as follows:

$$(H:P)_B = \{\{MW(Conjugate) - MW(Prot)\} / MW(Hapten)\}.$$

**Screening of the competitive As/CA combinations.** Preliminary evaluation of the avidity of antibodies for the antigens was made by measuring the binding of serial dilutions of the antisera **As112-As120** (dilutions from 1/1000 to 1/64000) to microtiter plates coated with each individual coating antigen (CA, 1μg $mL^{-1}$). Those combinations giving absorbance values higher than 0.5 for the more concentrated dilution of antibody after 30 min of competition were initially chosen

to evaluate the ability of the analyte (**$SPC_{MIX}$**) to shift the equilibrium of the binding of the different As/CA combination was evaluated by coating four rows of three consecutive columns of the microtiter plates with the same coating antigen at a fixed concentration (1 μg $mL^{-1}$). Solutions of **$SPC_{MIX}$** at **zero** (PBST), **high** (150 μM) and **low** (200 nM) concentration were added (50 μL/well) respectively to each consecutive column, followed by the addition of serial dilutions of the antiserum (four consecutive dilutions comprised between 1/1000 and 1/32000 in PBST depending on the results of the experiment above, 50 μL/well). The mixture was incubated for 30 min at room temperature, and the plates were processed as described in the general procedure. As/CA combinations showing an inhibition of the absorbance in the presence of SPC higher than 50% for the high concentration (150 μM) and higher than 15% for the low concentration (200 nM) were chosen for further studies on competitive assays. Inhibition was calculated according to the following formula:

$$(1-[\text{absorbance}(SPC_{MIX})/\text{absorbance}(\text{zero } SPC_{MIX}])\times 100$$

using PBST with 1% of BSA as competition buffer instead of PBST for the combination **As112/2$C_5$-OVA**.

**Two-Dimensional Checkerboard Titration Experiments.** The conditions of the assay regarding the concentration of the immunoreagents (As and CA) were established using two-dimensional checkerboard titration experiments by measuring the binding of serial dilutions of each sera (8 dilutions, 1/500-1/32000 in PBST and zero of As, 100 μL/well) to microtiters plates coated with different solutions of each CA (12 solutions, from 10 μg $mL^{-1}$ to 9 ng $mL^{-1}$ in coating buffer, 100 μL/well). Optimal concentrations for the antisera dilution and the concentration of the coating antigen were chosen to produce absorbances around 0.7-1 units of absorbance after 30 min of incubation at room temperature.

**Table A.** Results from the screening experiments of the SPC antisera[a]

| Antigen | | Antisera | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Type A | | | Type B | | | Type AB | | |
| | | 112 | 113 | 114 | 115 | 116 | 117 | 118 | 119 | 120 |
| Type A Antigens | $2C_3$-S-BSA | H | H | L | | | | H | H | L |
| | $2C_4$-S-BSA | H | M | L | | | | M | L | M |
| | $2C_5$-S-BSA | H | H | L | VH | VH | VH | H | H | L |
| | $3C_4$-S-BSA | H | M | L | VH | VH | VH | M | L | H |
| | $3C_5$-S-BSA | H | M | L | VH | | | M | L | L |
| | $5C_5$-S-BSA | H | H | L | | | | M | H | L |
| | $2C_4$-S-OVA | H | H | M | | | | M | L | M |
| | $2C_5$-S-OVA | H | H | L | VH | VH | | M | M | L |
| | $3C_4$-S-OVA | H | M | L | VH | | | H | L | M |
| | $3C_5$-S-OVA | M | M | M | | | | M | L | L |
| | $5C_5$-S-OVA | M | M | L | | | | M | H | L |
| | $2C_4$-S-CONA | H | M | L | | | | L | L | L |
| | $2C_5$-S-CONA | H | M | L | VH | VH | H | L | L | L |
| | $3C_4$-S-CONA | H | L | L | VH | VH | VH | M | L | M |
| | $3C_5$-S-CONA | H | L | L | VH | | | M | M | L |
| | $5C_5$-S-CONA | H | M | L | | | | M | H | L |
| Type B Antigens | $2C_3$-BSA | | | | VH | VH | VH | VH | VH | H |
| | $2C_4$-BSA | | | | VH | VH | VH | H | VH | VH |
| | $2C_5$-BSA | VH | | | VH | VH | H | H | VH | VH |
| | $3C_4$-BSA | VH | | | H | M | VH | VH | VH | M |
| | $3C_5$-BSA | VH | | | VH | M | H | VH | VH | H |
| | $3C_6$-BSA | VH | VH | | H | VH | M | M | VH | H |
| | $5C_5$-BSA | | | | VH | VH | M | H | VH | VH |
| | $9C_9$-BSA | | | | H | H | VH | VH | VH | H |
| | $2C_3$-OVA | | | | VH | H | H | H | H | H |
| | $2C_4$-OVA | | | | H | H | H | H | H | H |
| | $2C_5$-OVA | VH | VH | | M | M | H | M | M | M |
| | $3C_4$-OVA | VH | | | H | H | M | H | H | H |
| | $3C_5$-OVA | VH | H | | VH | M | H | M | M | M |
| | $3C_6$-OVA | H | VH | M | H | H | H | M | M | M |
| | $5C_5$-OVA | | | | VH | H | H | M | H | H |
| | $9C_9$-OVA | | | | VH | VH | VH | H | VH | H |

**Absorbance**
- **>1.5**
- **1.0-1.5**
- **0.5-1.0**
- **<0.5**

[a] Grey shadows define the avidity of the antibodies (dil 1/1000 in PBS) for the antigens coated on microtiter plates (1 μg $L^{-1}$ in coating buffer). The letter codes indicate the relative recognition of the SPCs under competitive conditions for each As/coating antigen combination. $SPC_{MIX}$ was used as standard analyte at two concentrations ($I_1$=150 μM and $I_2$= 200nM). The degree of inhibition produced is expressed as VH-Very high ($I_1$>80%, $I_2$>30%); H-High ($I_1$>50%, $I_2$>15%); M-Medium ($I_1$>50%, $I_2$<15%); L-Low ($I_1$<50%, $I_2$<15%).

**Table B.1.** Results from the two-dimensional checkerboard titration experiments. Type A antisera[a]

| Coating Antigen | As112 | | | As113 | | | As114 | | |
|---|---|---|---|---|---|---|---|---|---|
| | [AT][b] | As[c] | Abs. | [AT] | As | Abs. | [AT] | As | Abs. |
| **$2C_3$-S-BSA** | 0.078 | 1/160 | 0.831 | 0.078 | 1/20 | 0.896 | | | |
| **$2C_4$-S-BSA** | 0.156 | 1/320 | 1.049 | | | | | | |
| **$2C_4$-S-OVA** | 0.078 | 1/80 | 0.951 | 0.156 | 1/20 | 0.944 | 0.156 | 1/10 | 0.947 |
| **$2C_4$-S-CONA** | 0.078 | 1/160 | 0.758 | | | | | | |
| **$2C_5$-S-BSA** | 0.313 | 1/160 | 0.793 | 0.078 | 1/40 | 0.869 | | | |
| **$2C_5$-S-OVA** | 0.156 | 1/160 | 0.794 | 0.078 | 1/40 | 0.765 | | | |
| **$2C_5$-S-CONA** | 0.156 | 1/80 | 0.966 | 0.078 | 1/40 | 0.881 | | | |
| **$3C_4$-S-BSA** | 0.078 | 1/80 | 0.851 | | | | | | |
| **$3C_4$-S-OVA** | 0.156 | 1/80 | 1.073 | | | | | | |
| **$3C_4$-S-CONA** | 0.078 | 1/80 | 0.756 | | | | | | |
| **$3C_5$-S-BSA** | 0.156 | 1/80 | 0.759 | | | | | | |
| **$3C_5$-S-OVA** | 0.156 | 1/40 | 0.904 | | | | 0.156 | 1/20 | 0.865 |
| **$3C_5$-S-CONA** | 0.078 | 1/80 | 0.797 | | | | 0.078 | 1/40 | 0.843 |
| **$5C_5$-S-BSA** | 0.313 | 1/40 | 0.779 | 0.313 | 1/20 | 0.79 | | | |
| **$5C_5$-S-CONA** | 0.156 | 1/40 | 0.782 | | | | | | |
| **$2C_3$-BSA** | | | | | | | | | |
| **$2C_4$-BSA** | | | | | | | | | |
| **$2C_5$-BSA** | 0.313 | 1/5 | 0.603 | | | | | | |
| **$3C_4$-BSA** | 0.039 | 1/5 | 0.587 | | | | | | |
| **$3C_5$-BSA** | 0.625 | 1/5 | 0.672 | | | | | | |
| **$3C_6$-BSA** | 0.039 | 1/5 | 0.544 | 0.625 | 1/10 | 0.801 | | | |
| **$5C_5$-BSA** | | | | | | | | | |
| **$9C_9$-BSA** | | | | | | | | | |
| **$2C_3$-OVA** | | | | | | | | | |
| **$2C_4$-OVA** | | | | | | | | | |
| **$2C_5$-OVA** | 0.019 | 1/5 | 0.801 | 0625 | 1/5 | 0.934 | | | |
| **$3C_4$-OVA** | 0.039 | 1/5 | 0.832 | | | | | | |
| **$3C_5$-OVA** | 0.019 | 1/5 | 0.534 | 1.250 | 1/5 | 0.728 | | | |
| **$3C_6$-OVA** | | | | 0.313 | 1/10 | 0.781 | | | |
| **$5C_5$-OVA** | | | | | | | | | |
| **$9C_9$-OVA** | | | | | | | | | |

[a]. The blank rows indicate combinations not selected in the previous screening experiments and therefore not tested in the two-dimensional checkerboard titration experiments

[b] CA concentration expressed in μg $mL^{-1}$.

[c] Antisera dilution (x100)

**Table B.2.** Results from the two-dimensional checkerboard titration experiments. Type B antisera[a]

| Coating Antigen | As115 | | | As116 | | | As117 | | |
|---|---|---|---|---|---|---|---|---|---|
| | [AT][b] | As[c] | Abs. | [AT] | As | Abs. | [AT] | As | Abs. |
| **$2C_3$-S-BSA** | | | | | | | | | |
| **$2C_4$-S-BSA** | | | | | | | | | |
| **$2C_4$-S-OVA** | | | | | | | | | |
| **$2C_4$-S-CONA** | | | | | | | | | |
| **$2C_5$-S-BSA** | 0.625 | 1/40 | 0.989 | 0.156 | 1/20 | 0.795 | 0.625 | 1/5 | 0.426 |
| **$2C_5$-S-OVA** | 1.250 | 1/20 | 0.904 | 0.625 | 1/20 | 0.811 | | | |
| **$2C_5$-S-CONA** | 0.625 | 1/10 | 0.821 | 0.313 | 1/20 | 0.857 | | | |
| **$3C_4$-S-BSA** | 0.625 | 1/20 | 0.778 | 0.313 | 1/20 | 0.776 | 0.313 | 1/5 | 0.400 |
| **$3C_4$-S-OVA** | 1.250 | 1/10 | 0.741 | | | | | | |
| **$3C_4$-S-CONA** | 0.313 | 1/20 | 0.712 | 0.625 | 1/10 | 0.625 | 1.25 | 1/10 | 0.748 |
| **$3C_5$-S-BSA** | 1.250 | 1/5 | 0.417 | | | | | | |
| **$3C_5$-S-OVA** | | | | | | | | | |
| **$3C_5$-S-CONA** | 1.250 | 1/10 | 0.654 | | | | | | |
| **$5C_5$-S-BSA** | | | | | | | | | |
| **$5C_5$-S-CONA** | | | | | | | | | |
| **$2C_3$-BSA** | 0.313 | 1/80 | 0.800 | 0.313 | 1/160 | 0.722 | 0.416 | 1/80 | 0.800 |
| **$2C_4$-BSA** | 0.313 | 1/160 | 0.784 | 0.313 | 1/160 | 0.877 | 0.313 | 1/80 | 0.924 |
| **$2C_5$-BSA** | 0.313 | 1/160 | 0.781 | 0.313 | 1/80 | 0.995 | 0.313 | 1/80 | 0.839 |
| **$3C_4$-BSA** | 0.039 | 1/320 | 0.875 | 0.039 | 1/320 | 0.921 | 0.039 | 1/160 | 0.859 |
| **$3C_5$-BSA** | 0.078 | 1/160 | 0.919 | 0.078 | 1/320 | 0.691 | 0.039 | 1/80 | 1.013 |
| **$3C_6$-BSA** | 0.156 | 1/320 | 0.902 | 0.078 | 1/320 | 0.901 | 0.078 | 1/80 | 1.034 |
| **$5C_5$-BSA** | 0.156 | 1/160 | 0.828 | 0.156 | 1/160 | 1.036 | 0.078 | 1/80 | 0.765 |
| **$9C_9$-BSA** | 0.313 | 1/80 | 0.670 | 0.625 | 1/160 | 0.863 | 0.313 | 1/20 | 0.749 |
| **$2C_3$-OVA** | 0.156 | 1/640 | 0.807 | 0.039 | 1/640 | 0.969 | 0.039 | 1/40 | 0.852 |
| **$2C_4$-OVA** | 0.039 | 1/640 | 0.911 | 0.039 | 1/640 | 1.071 | 0.019 | 1/40 | 0.911 |
| **$2C_5$-OVA** | 0.019 | 1/160 | 0.917 | 0.078 | 1/640 | 1.069 | 0.039 | 1/40 | 0.879 |
| **$3C_4$-OVA** | 0.039 | 1/320 | 1.009 | 0.039 | 1/640 | 1.078 | 0.019 | 1/40 | 0.864 |
| **$3C_5$-OVA** | 0.039 | 1/160 | 1.075 | 0.019 | 1/640 | 0.902 | 0.039 | 1/40 | 0.945 |
| **$3C_6$-OVA** | 0.013 | 1/320 | 0.826 | 0.002 | 1/640 | 1.009 | 0.005 | 1/40 | 0.979 |
| **$5C_5$-OVA** | 0.156 | 1/160 | 0.981 | 0.156 | 1/320 | 0.814 | 0.078 | 1/20 | 0.865 |
| **$9C_9$-OVA** | 0.313 | 1/80 | 0.950 | 0.156 | 1/320 | 1.112 | 0.313 | 1/20 | 0.979 |

[a] The blank rows indicate combinations not selected in the previous screening experiments and therefore not tested in the two-dimensional checkerboard titration experiments

[b] CA concentration expressed in μg $mL^{-1}$.

[c] Antisera dilution (x100)

**Table B.3.** Results from the two-dimensional checkerboard titration experiments. Type AB antisera[a]

| Coating Antigen | As118 | | | As119 | | | As120 | | |
|---|---|---|---|---|---|---|---|---|---|
| | [AT][b] | As[c] | Abs. | [AT] | As | Abs. | [AT] | As | Abs. |
| **$2C_3$-S-BSA** | 0.208 | 1/10 | 0.750 | 0.104 | 1/20 | 0.760 | | | |
| **$2C_4$-S-BSA** | | | | | | | 0.625 | 1/10 | 0.915 |
| **$2C_4$-S-OVA** | | | | | | | | | |
| **$2C_4$-S-CONA** | | | | | | | | | |
| **$2C_5$-S-BSA** | 0.156 | 1/20 | 0.918 | 0.078 | 1/20 | 0.803 | | | |
| **$2C_5$-S-OVA** | | | | 0.156 | 1/10 | 0.856 | | | |
| **$2C_5$-S-CONA** | | | | | | | 0.156 | 1/10 | 0.926 |
| **$3C_4$-S-BSA** | 0.104 | 1/20 | 0.750 | | | | 0.313 | 1/10 | 0.877 |
| **$3C_4$-S-OVA** | 0.156 | 1/20 | 0.758 | | | | | | |
| **$3C_4$-S-CONA** | | | | | | | | | |
| **$3C_5$-S-BSA** | | | | | | | | | |
| **$3C_5$-S-OVA** | | | | | | | | | |
| **$3C_5$-S-CONA** | | | | 0.156 | 1/20 | 0.873 | 0.156 | 1/10 | 0.707 |
| **$5C_5$-S-BSA** | | | | 0.625 | 1/10 | 0.903 | | | |
| **$5C_5$-S-CONA** | | | | 0.156 | 1/10 | 0.806 | | | |
| **$2C_3$-BSA** | 0.313 | 1/40 | 0.792 | 0.313 | 1/40 | 0.918 | 0.625 | 1/10 | 0.669 |
| **$2C_4$-BSA** | 0.625 | 1/40 | 0.768 | 0.313 | 1/40 | 0.896 | 0.625 | 1/10 | 0.871 |
| **$2C_5$-BSA** | 0.313 | 1/40 | 0.963 | 0.313 | 1/40 | 0.900 | 0.078 | 1/20 | 0.806 |
| **$3C_4$-BSA** | 0.039 | 1/40 | 0.84 | 0.019 | 1/80 | 0.844 | 0.078 | 1/20 | 0.798 |
| **$3C_5$-BSA** | 0.039 | 1/40 | 1.009 | 0.039 | 1/80 | 1.049 | 0.078 | 1/10 | 0.902 |
| **$3C_6$-BSA** | 0.039 | 1/40 | 0.885 | 0.039 | 1/80 | 0.859 | 0.039 | 1/10 | 0.809 |
| **$5C_5$-BSA** | 0.078 | 1/20 | 0.932 | 0.039 | 1/40 | 1.089 | 0.313 | 1/10 | 0.735 |
| **$9C_9$-BSA** | 0.313 | 1/10 | 0.935 | 0.313 | 1/10 | 1.087 | 0.625 | 1/5 | 0.565 |
| **$2C_3$-OVA** | 0.078 | 1/10 | 0.969 | 0.019 | 1/40 | 0.907 | 0.078 | 1/10 | 0.954 |
| **$2C_4$-OVA** | 0.019 | 1/20 | 0.858 | 0.004 | 1/80 | 0.879 | 0.039 | 1/20 | 0.777 |
| **$2C_5$-OVA** | | | | 0.078 | 1/40 | 0.764 | | | |
| **$3C_4$-OVA** | 0.039 | 1/10 | 0.821 | 0.019 | 1/40 | 0.758 | 0.019 | 1/20 | 0.826 |
| **$3C_5$-OVA** | | | | 0.015 | 1/40 | 0.859 | | | |
| **$3C_6$-OVA** | | | | 0.004 | 1/20 | 0.904 | | | |
| **$5C_5$-OVA** | 0.156 | 1/10 | 0.822 | 0.078 | 1/20 | 0.927 | 0.313 | 1/10 | 0.801 |
| **$9C_9$-OVA** | 0.208 | 1/10 | 0.877 | 0.313 | 1/10 | 0.878 | 0.313 | 1/10 | 0.784 |

[a] The blank rows indicate combinations not selected in the previous screening experiments and therefore not tested in the two-dimensional checkerboard titration experiments

[b] CA concentration expressed in μg $mL^{-1}$.

[c] Antisera dilution (x100)

**Table C.** Features of all the competitive immunoassays selected for the three families of antisera raised against the three types of SPCs immunizing haptens[a]

| As | Coating Antigen | $A_{max}$ | $A_{min}$ | $IC_{50}$, μg $L^{-1}$[b] | slope | $r^2$ |
|---|---|---|---|---|---|---|
| **As112** | **$2C_3$-S-BSA** | 1.476 | 0.012 | 1055 | -0.629 | 0.992 |
| | **$2C_4$-S-BSA** | 0.785 | -1.211 | 500000 | -0.217 | 0.782 |
| | **$2C_5$-S-BSA** | 1.491 | 0.614 | 6748 | -1.525 | 0.506 |
| | **$3C_4$-S-BSA** | 1.412 | -0.438 | 29347 | -0.526 | 0.720 |
| | **$3C_5$-S-BSA** | 1.066 | -5.782 | 1500000 | -0.528 | 0.573 |
| | **$5C_5$-S-BSA** | 1.513 | -5.384 | 4000000 | -0.355 | 0.849 |
| | **$2C_4$-S-OVA** | 0.673 | 0.105 | 350 | -0.972 | 0.830 |
| | **$2C_5$-S-OVA** | 0.935 | -0.521 | 31667 | -0.382 | 0.702 |
| | **$3C_4$-S-OVA** | 0.977 | -3.838 | 2400000 | -0.452 | 0.643 |
| | **$3C_5$-S-OVA** | 1.101 | -2.781 | 237376 | -0.692 | 0.627 |
| | **$2C_4$-S-CONA** | 0.853 | 0.143 | 8761 | -27.63 | 0.615 |
| | **$2C_5$-S-CONA** | 1.291 | -1.363 | 325000 | -0.326 | 0.82 |
| | **$3C_4$-S-CONA** | ---- | ---- | ---- | ---- | ---- |
| | **$3C_5$-S-CONA** | ---- | ---- | ---- | ---- | ---- |
| | **$5C_5$-S-CONA** | 1.047 | -1.513 | 42000 | -9.015 | 0.715 |
| | **$2C_5$-OVA** | 0.818 | 0.087 | 1.14 | -0.932 | 0.998 |
| | **$3C_4$-OVA** | 0.699 | 0.089 | 0.99 | -1.048 | 0.996 |
| | **$3C_5$-OVA** | 0.803 | 0.067 | 1.65 | -1.036 | 0.992 |
| | **$2C_5$-BSA** | 0.350 | 0.059 | 1.31 | -1.929 | 0.872 |
| | **$3C_4$-BSA** | 0.585 | 0.046 | 1.58 | -2.047 | 0.922 |
| | **$3C_5$-BSA** | 0.776 | 0.059 | 6.58 | -1.345 | 0.927 |
| | **$3C_6$-BSA** | 0.831 | 0.041 | 3.18 | -1.679 | 0.982 |
| **As113** | **$2C_3$-S-BSA** | 1.145 | -7.972 | 68086 | -4.426 | 0.514 |
| | **$2C_5$-S-BSA** | 0.901 | -2.395 | 72860 | -2.504 | 0.848 |
| | **$5C_5$-S-BSA** | 0.855 | -10.43 | 89500 | -3.375 | 0.699 |
| | **$2C_4$-S-OVA** | 0.918 | -25.52 | 5000000 | -0.759 | 0.844 |
| | **$2C_5$-S-OVA** | 0.672 | -2.429 | 2250000 | -0.381 | 0.897 |
| | **$2C_5$-S-CONA** | ---- | ---- | ---- | ---- | ---- |
| | **$2C_5$-OVA** | 1.108 | 0.159 | 53.1 | -2.170 | 0.869 |
| | **$3C_5$-OVA** | 0.672 | 0.123 | 25.5 | -2.090 | 0.916 |
| | **$3C_6$-OVA** | 1.694 | 0.073 | 68.23 | -1.116 | 0.998 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | **$3C_6$-BSA** | 1.493 | 0.039 | 24.7 | -0.904 | 0.987 |
| **As114** | **$2C_4$-S-OVA** | ---- | ---- | ---- | ---- | ---- |
| | **$3C_5$-S-OVA** | ---- | ---- | ---- | ---- | ---- |
| | **$3C_5$-S-CONA** | 0.896 | -1.222 | 44170 | -11.9 | 0.795 |

**Table C.** *(Continued)*

| As | Coating Antigen | $A_{max}$ | $A_{min}$ | $IC_{50}$, mg $L^{-1}$[b] | slope | $r^2$ |
|---|---|---|---|---|---|---|
| **As115** | **$2C_5$-S-BSA** | 1.688 | 0 | 98.5 | -1.032 | 0.997 |
| | **$3C_4$-S-BSA** | 2.083 | -0.047 | 297 | -0.903 | 0.983 |
| | **$3C_5$-S-BSA** | 0.909 | 0.044 | 96.7 | -1.661 | 0.939 |
| | **$2C_5$-S-OVA** | 0.992 | 0.068 | 48.3 | -3.185 | 0.964 |
| | **$3C_4$-S-OVA** | 1.405 | 0.068 | 135 | -1.324 | 0.923 |
| | **$2C_5$-S-CONA** | 1.603 | -0.001 | 428 | -0.846 | 0.906 |
| | **$3C_4$-S-CONA** | 1.071 | 0.061 | 76.3 | -1.206 | 0.977 |
| | **$3C_5$-S-CONA** | 1.262 | 0.103 | 71 | -1.365 | 0.896 |
| | **$2C_3$-OVA** | 1.241 | -0.123 | 50.9 | -0.345 | 0.912 |
| | **$2C_4$-OVA** | 1.24 | 0.113 | 11.2 | -0.743 | 0.963 |
| | **$2C_5$-OVA** | 1.435 | -0.214 | 111 | -0.313 | 0.918 |
| | **$3C_4$-OVA** | 1.731 | -0.006 | 82.5 | -0.455 | 0.944 |
| | **$3C_5$-OVA** | 1.228 | -0.821 | 1275 | -0.248 | 0.959 |
| | **$3C_6$-OVA** | 1.629 | 0.101 | 10.5 | -0.608 | 0.972 |
| | **$5C_5$-OVA** | 0.9569 | -0.036 | 130 | -0.567 | 0.992 |
| | **$9C_9$-OVA** | 1.091 | -0.136 | 1507 | -0.436 | 0.806 |
| | **$2C_3$-BSA** | 0.813 | -0.087 | 497 | -0.539 | 0.991 |
| | **$2C_4$-BSA** | 0.916 | 0.011 | 195 | -0.601 | 0.978 |
| | **$2C_5$-BSA** | 0.881 | 0.021 | 132.7 | -0.831 | 0.985 |
| | **$3C_4$-BSA** | 1.345 | -0.019 | 83.2 | -0.367 | 0.984 |
| | **$3C_5$-BSA** | 1.173 | -0.123 | 1400 | -0.471 | 0.982 |
| | **$3C_6$-BSA** | 1.548 | -0.229 | 154.2 | -0.299 | 0.944 |
| | **$5C_5$-BSA** | 1.108 | -0.028 | 205 | -0.623 | 0.981 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | **$9C_9$-BSA** | 0.816 | 0.007 | 214.7 | -0.688 | 0.843 |
| **As116** | **$2C_5$-S-BSA** | 0.713 | 0.045 | 65 | -0.945 | 0.702 |
| | **$3C_4$-S-BSA** | 0.612 | 0.045 | 60.3 | -1.132 | 0.789 |
| | **$2C_5$-S-OVA** | 0.435 | 0.024 | 101 | -0.814 | 0.978 |
| | **$2C_5$-S-CONA** | 0.552 | 0.028 | 282 | -0.791 | 0.975 |
| | **$3C_4$-S-CONA** | 0.984 | 0.029 | 437 | -0.834 | 0.858 |
| | **$2C_3$-OVA** | 0.856 | 0.004 | 648 | -0.584 | 0.988 |
| | **$2C_4$-OVA** | 0.911 | -0.021 | 884 | -0.591 | 0.980 |
| | **$2C_5$-OVA** | 0.575 | 0.014 | 2352 | -1.034 | 0.700 |
| | **$3C_4$-OVA** | 0.418 | -0.259 | 17350 | -0.479 | 0.901 |
| | **$3C_5$-OVA** | 0.438 | -0.017 | 661 | -0.593 | 0.867 |
| | **$3C_6$-OVA** | 0.175 | 0.002 | 222 | -0.666 | 0.918 |
| | **$5C_5$-OVA** | 0.643 | -1.099 | 192000 | -0.371 | 0.855 |
| | **$9C_9$-OVA** | 0.425 | -0.025 | 750 | -0.583 | 0.924 |
| | **$2C_3$-BSA** | 0.567 | -0.038 | 457 | -0.421 | 0.897 |
| | **$2C_4$-BSA** | 0.769 | -0.011 | 245.9 | -0.563 | 0.955 |
| | **$2C_5$-BSA** | 0.753 | -0.007 | 217 | -0.556 | 0.941 |
| | **$3C_4$-BSA** | 0.824 | -2.104 | 2500000 | -0.219 | 0.849 |
| | **$3C_5$-BSA** | 0.869 | -0.164 | 3100 | -0.548 | 0.927 |
| | **$3C_6$-BSA** | 0.895 | -0.091 | 2548 | -0.664 | 0.864 |

**Table C.** *(continued)*

| As | Coating Antigen | $A_{max}$ | $A_{min}$ | $IC_{50}$, mg $L^{-1}$[b] | slope | $r^2$ |
|---|---|---|---|---|---|---|
| **As116** | **$5C_5$-BSA** | 0.948 | 0.023 | 3800 | -1.204 | 0.899 |
| | **$9C_9$-BSA,** | 0.725 | -0.057 | 1375 | -0.719 | 0.890 |
| **As117** | **$3C_4$-S-CONA** | 0.957 | 0.079 | 137 | -0.931 | 0.931 |
| | **$2C_3$-OVA** | 1.421 | 0.017 | 1448 | -0.832 | 0.978 |
| | **$2C_4$-OVA** | 1.031 | 0.002 | 2435 | -0.923 | 0.890 |
| | **$2C_5$-OVA** | 1.412 | 0.105 | 3500 | -1.132 | 0.813 |
| | **$3C_4$-OVA** | 0.953 | -0.165 | 4875 | -0.581 | 0.804 |
| | **$3C_5$-OVA** | 1.615 | -0.086 | 6500 | -1.043 | 0.845 |
| | **$3C_6$-OVA** | 0.743 | -0.088 | 1121 | -0.532 | 0.860 |
| | **$5C_5$-OVA** | 1.081 | -1.446 | 79000 | -0.409 | 0.907 |
| | **$9C_9$-OVA** | 1.062 | -0.332 | 5590 | -0.558 | 0.939 |

| | | | | | | |
|---|---|---|---|---|---|---|
| | **$2C_3$-BSA** | 0.641 | -0.007 | 400 | -0.764 | 0.957 |
| | **$2C_4$-BSA** | 0.791 | -0.025 | 286 | -0.611 | 0.956 |
| | **$2C_5$-BSA** | 0.799 | -0.004 | 264 | -0.689 | 0.897 |
| | **$3C_4$-BSA** | 0.711 | -0.018 | 842 | -0.722 | 0.936 |
| | **$3C_5$-BSA** | 1.221 | -0.033 | 1509 | -0.687 | 0.865 |
| | **$3C_6$-BSA** | 1.183 | -0.113 | 2313 | -0.566 | 0.915 |
| | **$5C_5$-BSA** | 0.841 | -0.069 | 860 | -0.631 | 0.972 |
| | **$9C_9$-BSA** | 0.759 | -0.013 | 369 | -0.931 | 0.978 |
| **As118** | **$2C_3$-S-BSA** | 1.214 | 0.454 | 27949 | -6.803 | 0.607 |
| | **$2C_5$-S-BSA** | 1.006 | -1.223 | 42216 | -8.397 | 0.711 |
| | **$3C_4$-S-BSA** | 0.839 | -1.895 | 49580 | -4.866 | 0.846 |
| | **$3C_4$-S-OVA** | 0.731 | -1.611 | 47700 | -5.109 | 0.864 |
| | **$2C_3$-OVA** | 1.151 | -4.497 | $2.35\ 10^5$ | -0.384 | 0.778 |
| | **$2C_4$-OVA** | 0.751 | -0.097 | 792 | -0.466 | 0.985 |
| | **$3C_4$-OVA** | 1.035 | -0.086 | 2312 | -0.679 | 0.895 |
| | **$5C_5$-OVA** | 0.972 | -0.191 | 4608 | -0.714 | 0.931 |
| | **$9C_9$-OVA** | 0.797 | -0.138 | 2825 | -0.654 | 0.954 |
| | **$2C_3$-BSA** | 0.993 | 0.011 | 198 | -0.674 | 0.993 |
| | **$2C_4$-BSA** | 1.424 | -0.024 | 705 | -0.704 | 0.982 |
| | **$2C_5$-BSA** | 1.129 | 0.001 | 252 | -0.696 | 0.956 |
| | **$3C_4$-BSA** | 1.231 | 0.011 | 422 | -0.723 | 0.825 |
| | **$3C_5$-BSA** | 1.332 | -0.051 | 430 | -0.576 | 0.931 |
| | **$3C_6$-BSA** | 1.373 | -0.083 | 767 | -0.632 | 0.930 |
| | **$5C_5$-BSA** | 1.273 | -0.203 | 2856 | -0.612 | 0.895 |
| | **$9C_9$-BSA** | 1.082 | 0.029 | 298 | -0.749 | 0.860 |
| **As119** | **$2C_3$-S-BSA** | ---- | ---- | ---- | ---- | ---- |
| | **$2C_5$-S-BSA** | ---- | ---- | ---- | ---- | ---- |
| | **$5C_5$-S-BSA** | ---- | ---- | ---- | ---- | ---- |
| | **$2C_5$-S-OVA** | ---- | ---- | ---- | ---- | ---- |
| | **$3C_5$-S-CONA** | ---- | ---- | ---- | ---- | ---- |
| | **$5C_5$-S-CONA** | ---- | ---- | ---- | ---- | ---- |

**Table C.** *(continued)*

| **As** | **Coating Antigen** | **$A_{max}$** | **$A_{min}$** | **$IC_{50}$, mg $L^{-1}$[b]** | **slope** | **$r^2$** |
|---|---|---|---|---|---|---|

| | | | | | | |
|---|---|---|---|---|---|---|
| **As119** | **2C$_3$-OVA** | 0.671 | 0.021 | 216 | -0.972 | 0.946 |
| | **2C$_4$-OVA** | 0.257 | -0.008 | 105 | -0.623 | 0.983 |
| | **2C$_5$-OVA** | 0.845 | -0.003 | 636 | -0.779 | 0.892 |
| | **3C$_4$-OVA** | 0.663 | -0.025 | 353 | -0.589 | 0.988 |
| | **3C$_5$-OVA** | 0.727 | -0.039 | 402 | -0.533 | 0.990 |
| | **3C$_6$-OVA** | 0.643 | -0.012 | 550 | -0.728 | 0.970 |
| | **5C$_5$-OVA** | 0.878 | -0.021 | 389 | -0.767 | 0.972 |
| | **9C$_9$-OVA** | 1.129 | -0.028 | 527 | -0.707 | 0.975 |
| | **2C$_3$-BSA** | 0.993 | 0.031 | 37 | -1.032 | 0.975 |
| | **2C$_4$-BSA** | 1.128 | 0.078 | 49 | -0.935 | 0.886 |
| | **2C$_5$-BSA** | 0.798 | 0.051 | 38 | -1.028 | 0.836 |
| | **3C$_4$-BSA** | 0.706 | 0.082 | 75 | -0.992 | 0.846 |
| | **3C$_5$-BSA** | 0.656 | 0.063 | 100 | -1.032 | 0.820 |
| | **3C$_6$-BSA** | 0.675 | 0.073 | 71 | -1.087 | 0.872 |
| | **5C$_5$-BSA** | 1.024 | 0.108 | 41 | -1.378 | 0.805 |
| | **9C$_9$-BSA** | 0.706 | 0.072 | 45 | -1.451 | 0.838 |
| **As120** | **2C$_4$-S-BSA** | ---- | ---- | ---- | ---- | ---- |
| | **3C$_4$-S-BSA** | 1.028 | -1.172 | 48250 | -5.934 | 0.889 |
| | **2C$_5$-S-CONA** | 0.988 | -4.338 | 25000000 | -1.127 | 0.902 |
| | **3C$_5$-S-CONA** | ---- | ---- | ---- | ---- | ---- |
| | **2C$_3$-OVA** | 0.808 | -0.072 | 2022 | -0.397 | 0.968 |
| | **2C$_4$-OVA** | 0.822 | -0.026 | 209 | -0.459 | 0.984 |
| | **3C$_4$-OVA** | 0.775 | -0.015 | 268 | -0.508 | 0.959 |
| | **5C$_5$-OVA** | 0.828 | -0.008 | 930 | -0.515 | 0.972 |
| | **9C$_9$-OVA** | 0.663 | -0.035 | 554 | -0.446 | 0.992 |
| | **2C$_3$-BSA** | 0.603 | -0.009 | 446 | -0.491 | 0.987 |
| | **2C$_4$-BSA** | 0.7661 | -0.051 | 721 | -0.526 | 0.995 |
| | **2C$_5$-BSA** | 0.182 | 0.007 | 65.1 | -0.766 | 0.941 |
| | **3C$_4$-BSA** | 0.906 | -0.089 | 1539 | -0.449 | 0.989 |
| | **3C$_5$-BSA** | 1.118 | -0.654 | 19080 | -0.353 | 0.983 |
| | **3C$_6$-BSA** | 1.002 | -0.001 | 1230 | -0.501 | 0.966 |
| | **5C$_5$-BSA** | 0.964 | -0.024 | 689 | -0.489 | 0.975 |
| | **9C$_9$-BSA** | 0.708 | 0.119 | 171 | -0.643 | 0.907 |

[a] The parameters were extracted from the four-parameter equation used to fit the standard curves. Each curve was built using two-well replicates.

[b] $SPC_{MIX}$ consisting on a equimolar mixture of the six short alkyl chain SPCs was used as standard analyte. An average molecular weight of 250 has been used to calculate the $IC_{50}$ in µg $L^{-1}$.

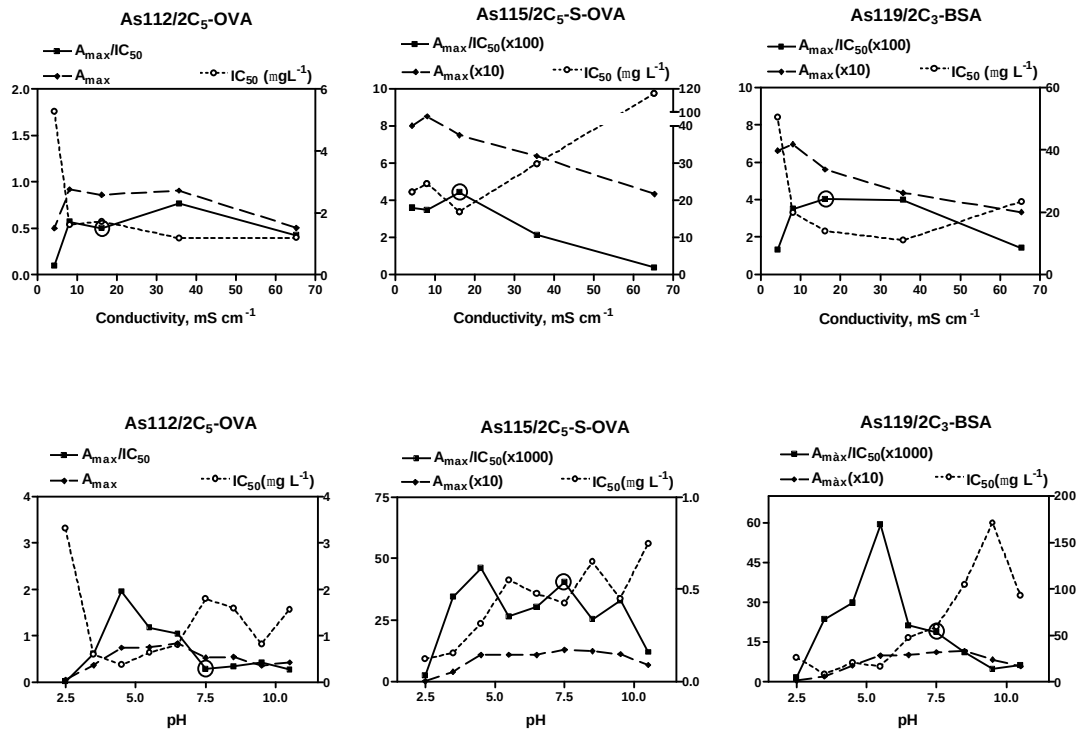


**Figure A:** Influence of the conductivity and the pH of the media on the three indirect SPC immunoassays (**As112/2$C_5$-OVA, As115/2$C_5$-S-OVA** and **As119/2$C_3$-BSA**). Left axes show the maximum absorbance ($A_{max}$) and the quotient of the $A_{max}$ and the $IC_{50}$ ($A_{max}/IC_{50}$). The right axes show the values of the $IC_{50}$ expressed in µg $L^{-1}$. The data shown have been extracted from the four-parameter equation used to fit the standard curves. Standard curves were prepared using two well replicates.