Table S1. Determination of coating antigen concentration and dilution factor of secondary antibody

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | | | **DS-Cav1 coating concentration** **(μg/mL)** | | | | | | | | | | | | Sample DF | |
| 10 | | 7.5 | | **5** | | 2.5 | | 1 | | 0 | |
| AP-conjugated anti-human IgG DF | **1:2000** | | 1:6000 | 2.7741 | 1.7662 | 2.4809 | 1.6319 | **2.4059** | 1.7617 | 1.8144 | 1.3032 | 1.0369 | 0.7494 | 0.2278 | 0.2016 | 1:100 |
| 1.6947 | 1.0093 | 1.4831 | 1.0247 | **1.4225** | 1.4176 | 1.0608 | 0.7942 | 0.5802 | 0.426 | 0.1092 | 0.1158 | 1:400 |
| 0.7664 | 0.5221 | 0.6824 | 0.5021 | **0.6734** | 0.5106 | 0.479 | 0.3949 | 0.2729 | 0.2107 | 0.0818 | 0.0827 | 1:1600 |
| 0.0715 | 0.0709 | 0.0747 | 0.0919 | **0.0725** | 0.0805 | 0.0708 | 0.6037 | 0.0708 | 0.0702 | 0.0697 | 0.0698 | PBS |
| **r2** | | | 0.9296 | 0.9440 | 0.9354 | 0.9268 | **0.9371** | 0.8453 | 0.9429 | 0.9512 | 0.9571 | 0.9582 | 0.9999 | 0.9950 |  |
| 1:4000 | | 1:8000 | 1.6704 | 1.6284 | 1.4697 | 1.5678 | 1.4603 | 1.747 | 1.1704 | 1.4225 | 0.6914 | 0.7186 | 0.1547 | 0.1749 | 1:100 |
| 1.0783 | 1.225 | 0.97 | 1.2021 | 1.1465 | 1.5979 | 0.7139 | 0.9101 | 0.3685 | 0.4341 | 0.0996 | 0.1086 | 1:400 |
| 0.5191 | 0.6651 | 0.4609 | 0.6946 | 0.6474 | 1.5051 | 0.3454 | 0.4583 | 0.2097 | 0.2054 | 0.0791 | 0.0886 | 1:1600 |
| 0.0708 | 0.0677 | 0.1356 | 0.151 | 0.3609 | 1.4337 | 0.0709 | 0.1775 | 0.0708 | 0.0775 | 0.0708 | 0.0791 | PBS |
| **r2** | | | 0.9163 | 0.8523 | 0.9216 | 0.8520 | 0.8830 | 0.9566 | 0.9358 | 0.9365 | 0.9682 | 0.9488 | 0.9956 | 0.9982 |  |
|  | | | | **SC-TM coating concentration (μg/mL)** | | | | | | | | | | | | Sample DF | |
| 10 | | 7.5 | | 5 | | **2.5** | | 1 | | 0 | |
| AP-conjugated anti-human IgG DF | **1:2000** | | 1:6000 | 2.8387 | 1.7967 | 2.7182 | 1.7786 | 2.652 | 1.8172 | **2.126** | 1.441 | 1.2594 | 1.0192 | 0.2436 | 0.2174 | 1:100 |
| 1.7711 | 1.0943 | 1.6214 | 1.1105 | 1.5419 | 1.1354 | **1.2207** | 0.8784 | 0.6791 | 0.7013 | 0.1124 | 0.1047 | 1:400 |
| 0.7899 | 0.5206 | 0.7126 | 0.4916 | 0.658 | 0.6246 | **0.5444** | 0.3997 | 0.3092 | 0.4742 | 0.0834 | 0.0806 | 1:1600 |
| 0.0865 | 0.0796 | 0.071 | 0.0709 | 0.0718 | 0.2498 | **0.072** | 0.0698 | 0.0707 | 0.1721 | 0.0694 | 0.0693 | PBS |
| **r2** | | | 0.9256 | 0.9330 | 0.9362 | 0.9277 | 0.9428 | 0.9433 | **0.9460** | 0.9350 | 0.9619 | 0.9051 | 0.9998 | 0.9998 |  |
| 1:4000 | | 1:8000 | 1.6484 | 1.6799 | 1.5593 | 1.601 | 1.5305 | 1.5315 | 1.3137 | 1.5005 | 0.7755 | 1.1824 | 0.1564 | 0.1628 | 1:100 |
| 1.0881 | 1.3165 | 1.0463 | 1.2929 | 1.0517 | 1.298 | 0.8156 | 1.0907 | 0.4945 | 0.7146 | 0.0977 | 0.1049 | 1:400 |
| 0.5102 | 0.6846 | 0.4741 | 0.7172 | 0.465 | 1.1113 | 0.3949 | 0.5752 | 0.2808 | 0.95 | 0.0779 | 0.0795 | 1:1600 |
| 0.0798 | 0.0892 | 0.0779 | 0.1023 | 0.2775 | 0.4798 | 0.0709 | 0.0753 | 0.2124 | 0.0911 | 0.0706 | 0.0746 | PBS |
| **r2** | | | 0.9120 | 0.8404 | 0.9084 | 0.8218 | 0.9276 | 0.7626 | 0.9297 | 0.8716 | 0.9661 | 0.7038 | 0.9978 | 0.9945 |  |
|  | | | | **G protein coating concentration (μg/mL)** | | | | | | | | | | | | Sample DF | |
| 10 | | 7.5 | | **5** | | 2.5 | | 1 | | 0  . | |
| AP-conjugated anti-human IgG DF | 1:2000 | **1:6000** | | 3.011 | 1.7195 | 2.8393 | 1.665 | 2.7137 | **1.5454** | 2.3192 | 1.3668 | 1.537 | 1.046 | 0.1698 | 0.1492 | 1:100 |
| 2.0961 | 1.1712 | 1.7733 | 1.0795 | 1.5666 | **0.9654** | 1.1828 | 0.7629 | 0.6004 | 0.4409 | 0.064 | 0.0662 | 1:400 |
| 1.1998 | 0.6661 | 0.9499 | 0.5395 | 0.7395 | **0.4216** | 0.4407 | 0.2835 | 0.2026 | 0.1471 | 0.0367 | 0.0381 | 1:1600 |
| 0.7952 | 0.4199 | 0.5949 | 0.2027 | 0.2384 | **0.1192** | 0.1828 | 0.0603 | 0.0533 | 0.0348 | 0.0258 | 0.0271 | PBS |
| **r2** | | | 0.9389 | 0.9419 | 0.9595 | 0.9323 | 0.9548 | **0.9356** | 0.9746 | 0.9555 | 0.9927 | 0.9880 | 0.9999 | 0.9975 |  |
| 1:4000 | 1:8000 | | 2.6963 | 1.6475 | 2.5196 | 1.5633 | 2.387 | 1.4626 | 2.1244 | 1.2552 | 1.3519 | 0.9052 | 0.1336 | 0.1971 | 1:100 |
| 1.788 | 1.1832 | 1.5941 | 1.067 | 1.4305 | 0.9407 | 1.0904 | 0.776 | 0.5674 | 0.4214 | 0.0876 | 0.0579 | 1:400 |
| 1.0323 | 0.7528 | 0.8727 | 0.6033 | 0.6792 | 0.4546 | 0.4265 | 0.3092 | 0.2166 | 0.1625 | 0.0374 | 0.0383 | 1:1600 |
| 0.5684 | 0.4139 | 0.4338 | 0.3118 | 0.2048 | 0.1074 | 0.0812 | 0.0638 | 0.0426 | 0.0418 | 0.0256 | 0.0291 | PBS |
| **r2** | | | 0.9423 | 0.9193 | 0.9482 | 0.9307 | 0.9461 | 0.9245 | 0.9680 | 0.9343 | 0.9881 | 0.9810 | 0.9426 | 0.9967 |  |

Abbreviations: AP, alkaline phosphatase; DF, dilution factor; PBS, phosphate buffered saline

Table S2. Determination of optimal blocking buffer

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | | **DS-Cav1 (5 μg/ml)** | | | | | | | | | | |
| **1:100** | **1:400** | | | **1:1600** | | **1:6400** | | **PBS** | | **r2** |
| AP-conjugated anti-human IgG (1:2000) | PBS-T | 2.1863 | 1.236 | | | 0.563 | | 0.2178 | | 0.0696 | | 0.955 |
| 2.1255 | 1.1619 | | | 0.5145 | | 0.2109 | | 0.0622 | |
| Average | 2.1559 | 1.19895 | | | 0.53875 | | 0.21435 | | 0.0659 | |
| 1% BSA/PBS-T | 2.0293 | 1.0965 | | | 0.5054 | | 0.1929 | | 0.0607 | | 0.9561 |
| 1.9722 | 1.1182 | | | 0.483 | | 0.2055 | | 0.0617 | |
| Average | 2.00075 | 1.10735 | | | 0.4942 | | 0.1992 | | 0.0612 | |
| 10% FBS/PBS-T | 1.9805 | 1.0823 | | | 0.4631 | | 0.1889 | | 0.064 | | 0.9608 |
| 1.9768 | 1.0511 | | | 0.472 | | 0.1896 | | 0.0625 | |
| Average | 1.97865 | 1.0667 | | | 0.46755 | | 0.18925 | | 0.06325 | |
| 5% skim milk/PBS-T | 1.8606 | 0.9758 | | | 0.418 | | 0.1763 | | 0.0636 | | 0.9623 |
| 1.9056 | 1.0426 | | | 0.4648 | | 0.1892 | | 0.069 | |
| Average | 1.8831 | 1.0092 | | | 0.4414 | | 0.18275 | | 0.0663 | |
|  | | **SC-TM (2.5 μg/ml)** | | | | | | | | | | |
| **1:100** | **1:400** | | | **1:1600** | | **1:6400** | | **PBS** | | **r2** |
| AP-conjugated anti-human IgG (1:2000) | PBS-T | 1.6844 | 1.1055 | | | 0.507 | | 0.1974 | | 0.0654 | | 0.9307 |
| 1.7375 | 1.0485 | | | 0.4605 | | 0.1842 | | 0.0623 | |
| Average | 1.71095 | 1.077 | | | 0.48375 | | 0.1908 | | 0.06385 | |
| 1% BSA/PBS-T | 1.6646 | 1.0178 | | | 0.4398 | | 0.1877 | | 0.0646 | | 0.9445 |
| 1.7225 | 0.9906 | | | 0.4502 | | 0.1804 | | 0.0604 | |
| Average | 1.69355 | 1.0042 | | | 0.445 | | 0.18405 | | 0.0625 | |
| 10% FBS/PBS-T | 1.5759 | 0.9836 | | | 0.4138 | | 0.1801 | | 0.0605 | | 0.938 |
| 1.6129 | 0.9713 | | | 0.435 | | 0.1773 | | 0.0599 | |
| Average | 1.5944 | 0.97745 | | | 0.4244 | | 0.1787 | | 0.0602 | |
| 5% skim milk/PBS-T | 1.5442 | 0.8907 | | | 0.4027 | | 0.1719 | | 0.0618 | | 0.9482 |
| 1.573 | 0.9209 | | | 0.4292 | | 0.1648 | | 0.0655 | |
| Average | 1.5586 | 0.9058 | | | 0.41595 | | 0.16835 | | 0.06365 | |
|  | | **G protein (5 μg/ml)** | | | | | | | | | | |
| **1:100** | | **1:400** | **1:1600** | | **1:6400** | | **PBS** | | **r2** | |
| AP-conjugated anti-human IgG (1:6000) | PBS-T | 1.4734 | | 0.8668 | 0.374 | | 0.2265 | | 0.0557 | | 0.9975 | |
| 1.4565 | | 0.803 | 0.3938 | | 0.2241 | | 0.1692 | |
| Average | 1.46495 | | 0.8349 | 0.3839 | | 0.2253 | | 0.11245 | |
| 1% BSA/PBS-T | 1.5394 | | 0.9335 | 0.447 | | 0.2291 | | 0.1358 | | 0.9925 | |
| 1.5562 | | 0.9857 | 0.4639 | | 0.2246 | | 0.1224 | |
| Average | 1.5478 | | 0.9596 | 0.45545 | | 0.22685 | | 0.1291 | |
| 10% FBS/PBS-T | 1.435 | | 0.8117 | 0.3355 | | 0.1328 | | 0.0493 | | 0.9975 | |
| 1.5384 | | 0.8139 | 0.3376 | | 0.1324 | | 0.0472 | |
| Average | 1.4867 | | 0.8128 | 0.33655 | | 0.1326 | | 0.04825 | |
| 5% skim milk/PBS-T | 1.4735 | | 0.8452 | 0.356 | | 0.1236 | | 0.0287 | | 0.9949 | |
| 1.4966 | | 0.853 | 0.3469 | | 0.1226 | | 0.0285 | |
| Average | 1.48505 | | 0.8491 | 0.35145 | | 0.1231 | | 0.0286 | |

Abbreviations: AP, alkaline phosphatase; PBS, phosphate buffered saline; BSA, bovine serum albumin; FBS, fetal bovine serum