Certificate of Analysis

SARS-CoV-2 Protein Microarray
(2-in-1 Protein/Peptide Array)

CDI LABS

Product Number:	CDICOV2-001.0
Lot Number:	CDICOV2_JUL3_2020
Date of Manufacture:	July 3, 2020
Expiration Date:	12 months (6 months for functional assays)
Storage:	-80˚C (see details below)

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Summary and Application:

The SARS-CoV-2 Protein Microarray (2-in-1 Protein/Peptide Array) provides a flexible platform for analysis of human serum for antibodies to SARS-CoV-2. The microarray contains both full-length proteins and peptides spanning the S1 and S2 proteins for both analysis of antibodies binding full-length proteins and determining epitope level granularity with the S1 and S2 protein BSA-conjugated 12-mer peptides.

Characteristics:

Format/Content: 14 sub-arrays (2X7 format). Each sub-array contains the following full length SARS-CoV-2 proteins (E-protein, N-protein, NSP1, NSP2, NSP4, NSP5, NSP7, NSP8, NSP9, NSP10, RdR, NSP14, NSP15, S1, RBD, S2) and 12-mer peptides (conjugated to BSA) covering the S1 and S2 proteins. *(see microarray layout, right)*

Note: Human ACE2 and UBE2D3 are also included on this array but are not intended for use in this version and should be removed from the final analysis.

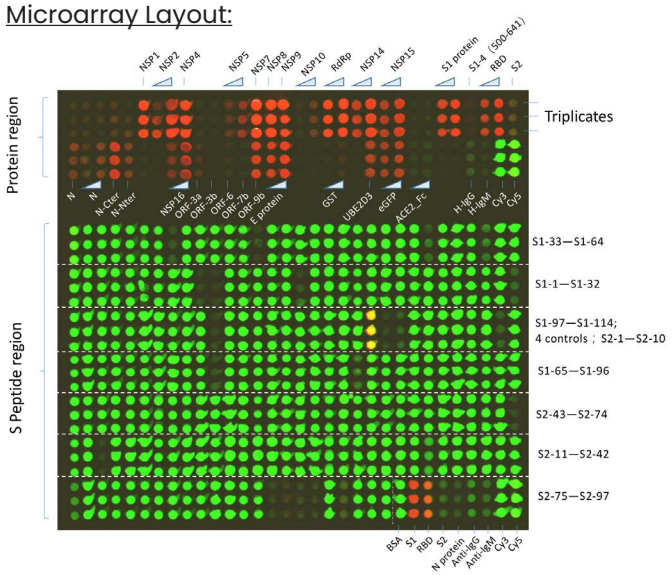
Manufacture: Non-contact piezoelectric inkjet on coated glass (PATH®); fluorescence detection

Protein Expression System: *E. coli*

Controls: Cy3, Cy5, BSA, eGFP, human IgG, human IgM, buffer only.

Storage: -80˚C in bags with plastic slide holders and desiccant.

Microarray Layout:



References:

1. Li Y *et al.,* Tao S (2020) **Linear epitope landscape of SARS-CoV-2 Spike protein constructed from 1,051 COVID-19 patients.** *medRxiv* preprint in review: https://doi.org/10.1101/2020.07.13.20152587; July 14. Click **here** to view.

2. Jiang H-w, Li Y, Zhang H-n, Wang W, Yang X, Qi H, Li H, Men D, Zhou J, Tao S-c (2020) **SARS-CoV-2 proteome microarray for global profiling of COVID-19 specific IgG and IgM responses.** *Nature Commun* **11**:Article number: 3581; July 14. Click **here** to view.

3. Li Y *et al.,* Tao S (2020) **Linear epitopes of SARS-CoV-2 spike protein elicit neutralizing antibodies in COVID-19 patients**; *medRxiv* preprint in review: https://doi.org/10.1101/2020.06.07.20125096; 7 Jun 2020. Click **here** to view.

Detailed Microarray Content:

Peptides

Peptide ID	AA Start	Sequence	AA End
S1-1	1	MFVFLVLLPLVS	12
S1-2	7	LLPLVSSQCVNL	18
S1-3	13	SQCVNLTTRTQL	24
S1-4	19	TTRTQLPPAYTN	30
S1-5	25	PPAYTNSFTRGV	36
S1-6	31	SFTRGVYYPDKV	42
S1-7	37	YYPDKVFRSSVL	48
S1-8	43	FRSSVLHSTQDL	54
S1-9	49	HSTQDLFLPFFS	60
S1-10	55	FLPFFSNVTWFH	66
S1-11	61	NVTWFHAIHVSG	72
S1-12	67	AIHVSGTNGTKR	78
S1-13	73	TNGTKRFDNPVL	84
S1-14	79	FDNPVLPFNDGV	90
S1-15	85	PFNDGVYFASTE	96
S1-16	91	YFASTEKSNIIR	102
S1-17	97	KSNIIRGWIFGT	108
S1-18	103	GWIFGTTLDSKT	114
S1-19	109	TLDSKTQSLLIV	120
S1-20	115	QSLLIVNNATNV	126
S1-21	121	NNATNVVIKVCE	132
S1-22	127	VIKVCEFQFCND	138
S1-23	133	FQFCNDPFLGVY	144
S1-24	139	PFLGVYYHKNNK	150
S1-25	145	YHKNNKSWMESE	156
S1-26	151	SWMESEFRVYSS	162
S1-27	157	FRVYSSANNCTF	168
S1-28	163	ANNCTFEYVSQP	174
S1-29	169	EYVSQPFLMDLE	180
S1-30	175	FLMDLEGKQGNF	186
S1-31	181	GKQGNFKNLREF	192
S1-32	187	KNLREFVFKNID	198
S1-33	193	VFKNIDGYFKIY	204
S1-34	199	GYFKIYSKHTPI	210
S1-35	205	SKHTPINLVRDL	216
S1-36	211	NLVRDLPQGFSA	222
S1-37	217	PQGFSALEPLVD	228
S1-38	223	LEPLVDLPIGIN	234
S1-39	229	LPIGINITRFQT	240
S1-40	235	ITRFQTLLALHR	246
S1-41	241	LLALHRSYLTPG	252
S1-42	247	SYLTPGDSSSGW	258
S1-43	253	DSSSGWTAGAAA	264
S1-44	259	TAGAAAYYVGYL	270
S1-45	265	YYVGYLQPRTFL	276
S1-46	271	QPRTFLLKYNEN	282
S1-47	277	LKYNENGTITDA	288
S1-48	283	GTITDAVDCALD	294
S1-49	289	VDCALDPLSETK	300
S1-50	295	PLSETKCTLKSF	306
S1-51	301	CTLKSFTVEKGI	312
S1-52	307	TVEKGIYQTSNF	318
S1-53	313	YQTSNFRVQPTE	324
S1-54	319	RVQPTESIVRFP	330
S1-55	325	SIVRFPNITNLC	336
S1-56	331	NITNLCPFGEVF	342
S1-57	337	PFGEVFNATRFA	348
S1-58	343	NATRFASVYAWN	354
S1-59	349	SVYAWNRKRISN	360
S1-60	355	RKRISNCVADYS	366
S1-61	361	CVADYSVLYNSA	372
S1-62	367	VLYNSASFSTFK	378
S1-63	373	SFSTFKCYGVSP	384
S1-64	379	CYGVSPTKLNDL	390
S1-65	385	TKLNDLCFTNVY	396
S1-66	391	CFTNVYADSFVI	402
S1-67	397	ADSFVIRGDEVR	408
S1-68	403	RGDEVRQIAPGQ	414
S1-69	409	QIAPGQTGKIAD	420
S1-70	415	TGKIADYNYKLP	426
S1-71	421	YNYKLPDDFTGC	432
S1-72	427	DDFTGCVIAWNS	438
S1-73	433	VIAWNSNNLDSK	444
S1-74	439	NNLDSKVGGNYN	450
S1-75	445	VGGNYNYLYRLF	456
S1-76	451	YLYRLFRKSNLK	462
S1-77	457	RKSNLKPFERDI	468
S1-78	463	PFERDISTEIYQ	474
S1-79	469	STEIYQAGSTPC	480
S1-80	475	AGSTPCNGVEGF	486
S1-81	481	NGVEGFNCYFPL	492
S1-82	487	NCYFPLQSYGFQ	498
S1-83	493	QSYGFQPTNGVG	504
S1-84	499	PTNGVGYQPYRV	510
S1-85	505	YQPYRVVVLSFE	516
S1-86	511	VVLSFELLHAPA	522
S1-87	517	LLHAPATVCGPK	528
S1-88	523	TVCGPKKSTNLV	534
S1-89	529	KSTNLVKNKCVN	540
S1-90	535	KNKCVNFNFNGL	546
S1-91	541	FNFNGLTGTGVL	552
S1-92	547	TGTGVLTESNKK	558
S1-93	553	TESNKKFLPFQQ	564
S1-94	559	FLPFQQFGRDIA	570
S1-95	565	FGRDIADTTDAV	576
S1-96	571	DTTDAVRDPQTL	582
S1-97	577	RDPQTLEILDIT	588
S1-98	583	EILDITPCSFGG	594
S1-99	589	PCSFGGVSVITP	600
S1-100	595	VSVITPGTNTSN	606
S1-101	601	GTNTSNQVAVLY	612
S1-102	607	QVAVLYQDVNCT	618
S1-103	613	QDVNCTEVPVAI	624
S1-104	619	EVPVAIHADQLT	630
S1-105	625	HADQLTPTWRVY	636
S1-106	631	PTWRVYSTGSNV	642
S1-107	637	STGSNVFQTRAG	648
S1-108	643	FQTRAGCLIGAE	654
S1-109	649	CLIGAEHVNNSY	660
S1-110	655	HVNNSYECDIPI	666
S1-111	661	ECDIPIGAGICA	672
S1-112	667	GAGICASYQTQT	678
S1-113	673	SYQTQTNSPRRA	684
S1-114	679	NSPRRARGGGGS	685
S2-1	686	SVASQSIIAYTM	697
S2-2	692	IIAYTMSLGAEN	703
S2-3	698	SLGAENSVAYSN	709
S2-4	704	SVAYSNNSIAIP	715
S2-5	710	NSIAIPTNFTIS	721
S2-6	716	TNFTISVTTEIL	727
S2-7	722	VTTEILPVSMTK	733
S2-8	728	PVSMTKTSVDCT	739
S2-9	734	TSVDCTMYICGD	745
S2-10	740	MYICGDSTECSN	751
S2-11	746	STECSNLLLQYG	757
S2-12	752	LLLQYGSFCTQL	763
S2-13	758	SFCTQLNRALTG	769
S2-14	764	NRALTGIAVEQD	775
S2-15	770	IAVEQDKNTQEV	781
S2-16	776	KNTQEVFAQVKQ	787
S2-17	782	FAQVKQIYKTPP	793
S2-18	788	IYKTPPIKDFGG	799
S2-19	794	IKDFGGFNFSQI	805
S2-20	800	FNFSQILPDPSK	811
S2-21	806	LPDPSKPSKRSF	817
S2-22	812	PSKRSFIEDLLF	823
S2-23	818	IEDLLFNKVTLA	829
S2-24	824	NKVTLADAGFIK	835
S2-25	830	DAGFIKQYGDCL	841
S2-26	836	QYGDCLGDIAAR	847
S2-27	842	GDIAARDLICAQ	853
S2-28	848	DLICAQKFNGLT	859
S2-29	854	KFNGLTVLPPLL	865
S2-30	860	VLPPLLTDEMIA	871
S2-31	866	TDEMIAQYTSAL	877
S2-32	872	QYTSALLAGTIT	883
S2-33	878	LAGTITSGWTFG	889
S2-34	884	SGWTFGAGAALQ	895
S2-35	890	AGAALQIPFAMQ	901
S2-36	896	IPFAMQMAYRFN	907
S2-37	902	MAYRFNGIGVTQ	913
S2-38	908	GIGVTQNVLYEN	919
S2-39	914	NVLYENQKLIAN	925
S2-40	920	QKLIANQFNSAI	931
S2-41	926	QFNSAIGKIQDS	937
S2-42	932	GKIQDSLSSTAS	943
S2-43	938	LSSTASALGKLQ	949
S2-44	944	ALGKLQDVVNQN	955
S2-45	950	DVVNQNAQALNT	961
S2-46	956	AQALNTLVKQLS	967
S2-47	962	LVKQLSSNFGAI	973
S2-48	968	SNFGAISSVLND	979
S2-49	974	SSVLNDILSRLD	985
S2-50	980	ILSRLDKVEAEV	991
S2-51	986	KVEAEVQIDRLI	997
S2-52	992	QIDRLITGRLQS	1003
S2-53	998	TGRLQSLQTYVT	1009
S2-54	1004	LQTYVTQQLIRA	1015
S2-55	1010	QQLIRAAEIRAS	1021
S2-56	1016	AEIRASANLAAT	1027
S2-57	1022	ANLAATKMSECV	1033
S2-58	1028	KMSECVLGQSKR	1039
S2-59	1034	LGQSKRVDFCGK	1045
S2-60	1040	VDFCGKGYHLMS	1051
S2-61	1046	GYHLMSFPQSAP	1057
S2-62	1052	FPQSAPHGVVFL	1063
S2-63	1058	HGVVFLHVTYVP	1069
S2-64	1064	HVTYVPAQEKNF	1075
S2-65	1070	AQEKNFTTAPAI	1081
S2-66	1076	TTAPAICHDGKA	1087
S2-67	1082	CHDGKAHFPREG	1093
S2-68	1088	HFPREGVFVSNG	1099
S2-69	1094	VFVSNGTHWFVT	1105
S2-70	1100	THWFVTQRNFYE	1111
S2-71	1106	QRNFYEPQIITT	1117
S2-72	1112	PQIITTDNTFVS	1123
S2-73	1118	DNTFVSGNCDVV	1129
S2-74	1124	GNCDVVIGIVNN	1135
S2-75	1130	IGIVNNTVYDPL	1141
S2-76	1136	TVYDPLQPELDS	1147
S2-77	1142	QPELDSFKEELD	1153
S2-78	1148	FKEELDKYFKNH	1159
S2-79	1154	KYFKNHTSPDVD	1165
S2-80	1160	TSPDVDLGDISG	1171
S2-81	1166	LGDISGINASVV	1177
S2-82	1172	INASVVNIQKEI	1183
S2-83	1178	NIQKEIDRLNEV	1189
S2-84	1184	DRLNEVAKNLNE	1195
S2-85	1190	AKNLNESLIDLQ	1201
S2-86	1196	SLIDLQELGKYE	1207
S2-87	1202	ELGKYEQYIKWP	1213
S2-88	1208	QYIKWPWYIWLG	1219
S2-89	1214	WYIWLGFIAGLI	1225
S2-90	1220	FIAGLIAIVMVT	1231
S2-91	1226	AIVMVTIMLCCM	1237
S2-92	1232	IMLCCMTSCCSC	1243
S2-93	1238	TSCCSCLKGCCS	1249
S2-94	1244	LKGCCSCGSCCK	1255
S2-95	1250	CGSCCKFDEDDS	1261
S2-96	1256	FDEDDSEPVLKG	1267
S2-97	1262	EPVLKGVKLHYT	1273

Full-length Proteins and Controls

E-protein_0.17 mg/ml	ORF-3b_0.05 mg/ml
E-protein_0.5 mg/ml	ORF-7b_0.05 mg/ml
N-Cter_0.25 mg/ml	ORF6_0.05 mg/ml
N-Nter_0.25 mg/ml	ORF-9b_0.2 mg/ml
N-protein_0.08 mg/ml	RBD_0.17 mg/ml
N-protein_0.25 mg/ml	RBD_0.5 mg/ml
N-protein_0.25 mg/ml	RdRp_0.17 mg/ml
NSP1_0.13 mg/ml	RdRp_0.5 mg/ml
NSP2_0.17 mg/ml	S1_0.17 mg/ml
NSP2_0.5 mg/ml	S1_0.5 mg/ml
NSP4_0.1 mg/ml	S1-4_0.2 mg/ml
NSP5_0.17 mg/ml	S2-1_0.1 mg/ml
NSP5_0.5 mg/ml	
NSP7_0.13 mg/ml	BSA
NSP8_0.25 mg/ml	ACE2_Fc_0.17 mg/ml
NSP9_0.25 mg/ml	ACE2_Fc_0.5 mg/ml
NSP10_0.17 mg/ml	Cy3
NSP10_0.5 mg/ml	Cy5
NSP14_0.17 mg/ml	eGFP_0.17 mg/ml
NSP14_0.5 mg/ml	eGFP_0.5 mg/ml
NSP15_0.17 mg/ml	GST_0.17 mg/ml
NSP15_0.5 mg/ml	GST_0.5 mg/ml
NSP16_0.17 mg/ml	Human IgG
NSP16_0.5 mg/ml	Human IgM
ORF-3a_0.05 mg/ml	UBE2D3-His-biotin

User Protocol >>>

User Protocol

SARS-CoV-2 Protein Microarray
(2-in-1 Protein/Peptide Array)

CDI LABS

Application Notes:

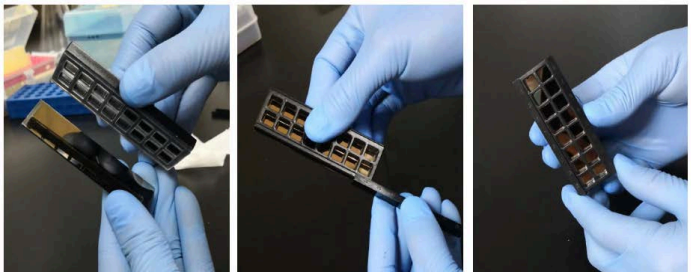
- Total *E. coli* Lysate is needed for serum profiling to prevent potential non-specific binding of the antibodies in serum against potential bacterial components, as protein contained in this array was produced in a *E. coli* expression system.
- The gasket can be reused. It should be thoroughly washed using detergent followed by clean water and air-dried after use.
- In steps 4-6, the slides should be shielded from light.
- Materials needed (not provided)

1. ProPlate® Multi-Well Slide Module (gracebio.com/product/pro-plate-multi-well-chambers-244864)
2. *E. coli* lysate (e.g. BioRad *E. coli* protein lysate 1632110 (www.bio-rad.com/en-us/product/e-coli-lysate-sample?ID=0e1226fb-c003-479c-8f25-057df2c7eff4).
3. Plastic 4-well plates to store the protein microarrays during the blocking, reaction and washing steps (e.g. Thermo Scientific *Nunc* Dishes, Rectangular 4-Well, No.12-565-495), or use a larger container
4. Aluminum foil.

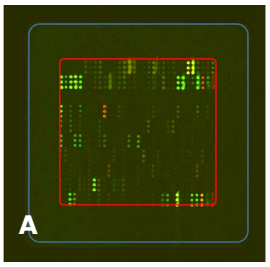
Protocol for Serum Profiling:

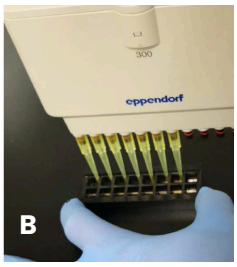
1. **Blocking.** The SARS-CoV-2 protein microarrays have been blocked prior to shipment.

2. **Incubation of sera.** Place the 16-chamber rubber gasket onto each slide to create individual chambers for the 14 identical sub-arrays (below) NOTE: The last two sub-arrays are intended to be blank.



Attach the gasket to slide and place the clips on both sides.





A. The relative size of the microarray area and the chamber. (Blue: the size of the chamber; Red: the microarray region)

B. Multi-channel pipette could be used for sample operation and liquid handling.

NOTE: Keep the pipette tips at one corner of the chamber to avoid touching the surface of the microarray region when pipetting.

Dilute sera (1:200) in PBS buffer containing 0.1% Tween 20, 1% BSA and 0.5 mg/ml total *E. coli* lysate. Then add 200 µl of diluted serum to each of the 14 separate sub-array wells and incubate at room temperature for 2 hours.

3. **Washing.** Wash each sub-array separately three times with PBS containing 0.1% Tween 20, with a multiple-channel pipette (pumping pipette plunger 20-30 times each is recommended to help wash thoroughly) to avoid contamination among samples. Remove the gasket from the slide. Transfer the slide to a larger container and wash three times (10 min each, at a speed of 100-110 rpm) using an orbital shaker.

4. **Incubation of the secondary antibody.** Dilute secondary antibodies (according to manufactures recommended dilution) in PBS buffer containing 0.1% Tween 20 and 1% BSA and incubate at room temperature for 1 hour.

   NOTE: Recommended secondary antibodies: Cy3-conjugated goat anti-human IgG (109-165-008; www.jacksonimmuno.com/catalog/products/109-165-008) and Alexa Fluor 647-conjugated donkey anti-human IgM (709-605-073); www.jacksonimmuno.com/catalog/products/709-605-073.

   Place arrays in 4-well microarray plate and add secondary, then cover with aluminum foil and incubate for 1 hour with gentle shaking.

5. **Washing.** Wash three times for 5 min. with PBS containing 0.1% Tween 20 on an orbital shaker (10 min, a speed of 100-110 rpm is recommended). Wash buffer: PBS with 0.1% Tween 20.
   And 2 additional washes with ddH_2O (5 min. each).

6. **Dry.** Once slides are dry, they are ready to scan.