SMC™ Human SARS-CoV-2 RBD IgG Kit

Microparticle Assay

Catalog # 03-0193-00

For the Qualitative Determination of SARS-CoV-2 RBD IgG in Human EDTA Plasma and Serum

FOR RESEARCH USE ONLY

NOT FOR USE IN DIAGNOSTIC PROCEDURES

Manufactured & Distributed by:

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INTRODUCTION

The Single Molecule Counting (SMC™) Human SARS-CoV-2 RBD IgG kit uses a fluorescent sandwich immunoassay technique to measure SARS-CoV-2 RBD IgG in Human EDTA Plasma and Serum samples. A Human SARS-CoV-2 RBD capture protein has been pre-coated onto paramagnetic microparticles (beads). The user pipettes beads and samples into uncoated microplate wells. During incubation, the SARS-CoV-2 RBD IgG present in the sample binds to the capture protein on the coated beads. Unbound molecules are washed away during the subsequent wash steps. Fluor-labeled detection antibody is added to each well and incubated. This detection antibody recognizes and binds to IgG antibodies against SARS-CoV-2 RBD that have been captured onto the beads, thus completing the immunosandwich. Elution buffer is then added and incubated. The elution buffer dissociates the bound protein sandwich from the beads surface releasing the labeled antibodies. These antibodies are separated during transfer to a final microplate. The plate is loaded into the SMCxPRO™ System where the labeled molecules are detected and counted. The number of fluor-labeled detection antibodies counted is directly proportional to the amount of SARS-CoV-2 RBD IgG present in the sample when captured. The amount of SARS-CoV-2 RBD IgG in unknown samples is qualitatively assessed in raw signal to determine COVID-19 infection when compared against known negative samples.

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SUPPLIES

The SMC™ Human SARS-CoV-2 RBD IgG Kit includes all reagents listed in *Table 1: Reagents Provided*. Additional reagents and supplies are required to run this immunoassay and are listed in *Table 2: Additional Supplies Required (not provided)*. All reagents supplied are for Research Use Only.

Table 1: Reagents Provided

Item #	Description	Shipping Conditions	Storage Conditions	Component Part No.	Packaging Details
1	Assay Buffer	With cold pack	2 - 8°C	02-0632-00	2 x 15 mL
2	SARS-CoV-2 RBD IgG Beads	With cold pack	2 - 8°C	02-2193-00	1 x 550 µL
3	Standard Diluent	With cold pack	2 - 8°C	02-0015-02	2 x 15 mL
4	SARS-CoV-2 RBD IgG Detection	With cold pack	2 - 8°C	02-1193-00	1 x 20 µL
5	10X Wash Buffer	With cold pack	2 - 8°C	02-0001-03	1 x 50 mL
6	Buffer D	With cold pack	2 - 8°C	02-0446-00	1 x 6 mL
7	Elution Buffer B	With cold pack	2 - 8°C	02-0211-02	1 x 5 mL

Storage Instructions

The SMC™ Human SARS-CoV-2 RBD IgG Kit should be stored at 2 - 8°C.

Supplied 10X Wash Buffer does not contain preservative. After dilution, the 1X Wash Buffer may be filter sterilized with Stericup® filter, EMD Millipore PN S2GPU11RE for storage of up to 1 month at 2 - 8°C. If not filter sterilized, all remaining 1X wash buffer should be discarded upon experiment completion.

Proper kit performance can only be guaranteed if the materials are stored properly.

Table 2: Additional Supplies Required (not provided)

Instrumentation

Item #	Product Description	Supplier	Product Number	Product Uses
1	12-Channel Manual Pipette 10 – 20 µL	--	--	Transferring 10 µL
2	12-Channel Manual Pipette 20 – 250 µL	--	--	Transferring 20 µL, 100 µL
3	Tube Rotator	--	--	Microparticle resuspension
4	Sphere Mag Plate	EMD Millipore	90-0003-02	Capturing/pelleting microparticles
5	Jitterbug™ Microplate Incubator/Shaker	EMD Millipore	70-0009-00	Incubating/Shaking at 25°C
6	VWR® Microplate Shaker	VWR International	12620-926	Plate shaking for overnight incubation, if recommended
7	Bio-Tek ELx™ 405 Microplate Washer	EMD Millipore	95-0004-05	Automated plate washing option
8	Tecan Hydroflex™ Microplate Washer	EMD Millipore	95-0005-02	Automated plate washing option
9	Centrifuge able to reach speed of 1,100 x g	--	--	Centrifuging samples, plates
10	Micro-Centrifuge	--	--	Centrifuge samples, provided Detection Antibody
11	VistaLab™ 25 mL Reservoirs	Fisher Scientific	21-381-27C	Addition of Reagents
12	96-well V-bottom plate	Fisher Scientific	14-222-241	Assay plate
13	5 mL Luer-Lok™ Syringe	Fisher Scientific	14-829-45	Detection Antibody filtration
14	0.2 µm Syringe Filter	EMD Millipore	SLGPR33RS	Detection Antibody filtration
15	Nunc™ Clear Adhesive Plate Seal	Fisher Scientific	236366	Sealing assay plate

Additional Supplies Required (not provided) continued

Materials

Item #	Product Description	Supplier	Product Number	Product Uses
16	SMCxPRO™ 384-well plate, 1 plate with adhesive seal	EMD Millipore	02-1008-00	SMCxPRO™ reading plate, seal
17	SMCxPRO™ 384-well plate, case of 32	Edition Eight, LLC	ABB2-00160A	SMCxPRO™ reading plate
18	SMCxPRO™ aluminum adhesive plate seals	Fisher Scientific	276014	SMCxPRO™ reading plate seals
19	Plate Roller	Fisher Scientific	NC9185793	Creates secure/even seal for each well of SMCxPRO™ reading plate
20	Universal plate cover	Fisher Scientific	253623	Covers assay plate
21	500 mL Container	--	--	Wash Buffer Dilution
22	Micro-centrifuge tubes	--	--	Sample storage, detection pre-dilution

Additional Reagents Required (not provided) continued

Reagents

Item #	Product Description	Supplier	Product Number	Product Uses
23	SMC™ 10X Wash Buffer (1 L)	EMD Millipore	02-0111-00	Automated plate washing

Please contact your technical services representative for additional information or assistance selecting required but not provided supplies.

TECHNICAL HINTS

To obtain reliable and reproducible results, the operator should carefully read this entire manual and fully understand all aspects of each assay step before running the assay. The following notes should be reviewed and understood before the assay is set up.

Assay Hints

1. Wipe down bench and pipettes with 70% isopropanol before use.
2. It is important to allow all reagents to warm to room temperature (20 - 25°C).
3. Use sterile filter pipette tips and reagent trays to avoid contamination.
4. Pre-wet tips (aspirate and dispense within well) twice before each transfer.
5. All washing must be performed with the wash buffer provided.
6. The recommended plate shaker settings are between #3 - #7 to provide maximal orbital mixing without splashing liquid or causing cross-contamination.
7. After the assay is complete, the plate should be read immediately.
    a. For SMCxPRO™ Immunoassay System – use adhesive seal.
8. The plates may be stored at 2-8°C for up to 48 hours away from light if same day reading is not possible.
    a. After the assay is complete, seal the plate before storing at 2 - 8°C
        i. For SMCxPRO™ Immunoassay System – use aluminum adhesive plate seal
    b. Bring to RT then centrifuge the plate at 1,100 *x g* for 1 minute prior to reading.

Instrument Hints

9. For optimal SMCxPRO™ performance, perform ASSIST testing on a daily basis (ideally at beginning of the day before assay is prepared).

PRECAUTIONS

- Use caution when handling biological samples. Wear protective clothing and gloves.
- Components of this reagent kit contain approximately 0.08% sodium azide as a preservative. Sodium azide is a toxic and dangerous compound when combined with acids or metals. Solutions containing sodium azide should be disposed of properly.

Full Hazard label:

Ingredient, Cat #		Full Label	
10X Wash Buffer	02-0001-03		**Warning.** Causes serious eye irritation. Harmful to aquatic life with long lasting effects. Avoid release to the environment. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
SARS-CoV-2 RBD IgG Coated Beads	02-2193-00		Harmful to aquatic life.
Assay Buffer	02-0632-00		Toxic to aquatic life. Harmful to aquatic life with long lasting effects. Avoid release to the environment.

ASSAY PREPARATION

Reagent Preparation

1. Warm all reagents to room temperature (RT) prior to use.
2. Store the Detection Antibody away from light until ready to use.
3. Prepare 1X Wash Buffer (from 10X Wash Buffer) as follows:
    a. Pour 50 mL of 10X Wash Buffer into a container capable of holding at least 500 mL. Add 450 mL of deionized water.
    b. Mix thoroughly by gentle inversion or with a clean, sterile stir bar.

    NOTE: 1X Wash Buffer may be filter sterilized *(refer to Storage Instructions)*
4. Mix SARS-CoV-2 RBD IgG Coated Beads on a rotisserie spin rotator, or manually by repeat inversion, for ≥ 20 minutes until all beads are resuspended.

Sample Preparation

1. Sample dilution
    - Dilute the samples 1:50 using the Standard Diluent *(e.g. for triplicates, transfer 10 µL of sample to the sample preparation plate and 490 µL Standard Diluent).*
    - 100 µL per well of 1:50 diluted EDTA plasma or Serum should be used.
        i. Futher dilutions have not been verified. Some samples may have raw data exceeding the software's range and will need a repeat at a higher dilution.

ASSAY PROCEDURE

Target Capture

1. Pipette 100 µL per well of 1:50 diluted Samples to assay plate.
2. Following mixing of the coated beads, immediately before adding to the assay plate, add the entire vial of coated Beads to 11.0 mL of supplied Assay Buffer. Rinse bead vial with 0.55 mL of Assay Buffer and ensure that all beads have been transferred. Mix by gentle inversion. There should be a total volume of 12.1 mL of diluted Coated Beads.
3. Pipette 100 µL per well of the Coated Beads into assay plate.
4. Seal assay plate with clear adhesive plate seal, apply pressure to seal to prevent leaking and cross-contamination.
5. Incubate for 1 hour at 25°C on microplate incubator/shaker (Jitterbug setting #4).
6. Approximately 10 minutes prior to the end of target capture incubation, prepare the detection antibody with following dilution steps:
    a. Dilute stock detection antibody 1:100 by adding 10 µL stock detection into 990 µL of Assay Buffer.
    b. Prepare an additional 1:10 dilution by adding 100 µL of 1:100 antibody into 900 µL of Assay Buffer. This is a final dilution of 1:1000.
    c. Prepare 1X detection antibody by adding 250 µL of 1:1000 diluted detection antibody into 4,750 µL of Assay Buffer and filter the diluted detection antibody using the syringe with a 0.2 µm filter into a clean tube.
7. When capture incubation is complete, centrifuge the assay plate at 1,100 *x g* for 1 minute and carefully remove clear adhesive plate seal to avoid splashing.

Post-Capture Wash

Wash plate once with a plate washer.

Plate Washer

a. BioTek; Post Capture Wash (POSTCAP) or
b. HydroFlex; Post Capture Wash (PCW)

If using automation please contact your technical service representative for the appropriate automation procedure.

ASSAY PROCEDURE (continued)

Detection

1. After removal from plate washer, dispense 20 µL per well of Detection Antibody without disturbing the bead pellet. *(It is recommended to change tips)*
2. Seal assay plate with clear adhesive plate seal.
3. Incubate for 0.5 hour at 25°C on microplate incubator/shaker (Jitterbug setting #5).
4. After incubation, carefully remove clear adhesive plate seal to avoid splashing.

Post-Detection Wash

Wash assay plate 4 times with a plate washer.

Plate Washer

a. BioTek; 4 cycle Pre-Transfer (4CYCPRE) or
b. HydroFlex; 4 cycle Pre-Transfer (*4cyPrTra)*

If using automation please contact your technical service representative for the appropriate automation procedure.

Post-Detection Shake

1. After 4 cycle Pre-Transfer wash, visually verify that each well contains ~200 µL of wash buffer.
2. Seal assay plate with clear adhesive plate seal and apply pressure to the seal to prevent leaking and cross-contamination.
3. Place plate on microplate/incubator shaker for 2 minutes (Jitterbug setting #3)
4. Remove the plate from the Jitterbug, carefully remove clear adhesive plate seal to avoid splashing and place it on the plate washer to perform Final Aspiration.

Final Aspiration

Plate Washer

a. BioTek; Final Aspirate (FINASP)
b. HydroFlex; Final Aspirate (FA_V1)

Elution

1. Dispense 10 µL Elution Buffer B per well using reverse pipetting without disturbing the bead pellet. *(It is recommended to change tips)*
2. Seal assay plate with a clear adhesive plate seal
3. Incubate plate for 10 minutes at 25°C on microplate incubator/shaker (Jitterbug setting #5).

ASSAY READING

To read on the SMCxPRO™ Immunoassay System

1. Add 10 µL per well of Buffer D using reverse pipetting to the SMCxPRO™ read plate (EMD Millipore PN 02-1008-00), using a 12-channel manual P20. The read plate should have the lid/plate holder placed on the bottom to protect the surface from scratching.
2. Place assay plate with Elution Buffer B onto sphere mag plate and allow beads to form a tight pellet for 2 minutes.
3. While keeping the assay plate containing eluate on sphere mag plate, gently remove clear adhesive seal and transfer 10 µL of eluate to the read plate containing Buffer D by aspirating directly from the V-bottom of the plate, avoiding the pelleted beads, and changing tips with each dispensed row.
4. Seal this plate with a clear adhesive plate seal.
5. Place the protected read plate (containing eluted, neutralized antibody solution) into Jitterbug and shake for 2 minutes at 25ºC (Jitterbug setting #5), centrifuge plate for 1 minute at RT, approximately 1,100 X g.
6. Seal reading plate with clear adhesive plate seal. Centrifuge plate for 1 minute at RT, approximately 1,100 *x g*. Remove plate sealer, inspect reading plate wells and remove bubbles if they are present. Reseal with aluminum adhesive seal.
7. Remove the plate holder from the sealed reading plate and load it onto the SMCxPRO™ Immunoassay System. Start read.

Note: There is a smart warm up period of up to 30 minutes to equilibrate plate temperature to internal instrument temperature. Once achieved the read will start automatically.

APPENDIX A: SMC™ Quick Assay Guide

1. Prepare all reagents and samples as instructed.
2. Add 100 µL of 1:50 diluted samples and 100 µL of **Coated Beads** to **assay plate**.
3. Seal and incubate for 1 hour at 25°C on appropriate microplate incubator/shaker.

1 hour 25°C

4. After capture incubation, centrifuge **assay plate** at 1,100 *x g* for 1 minute.
5. Perform Post-Capture Wash**.**
6. Remove from washer magnet and add 20 µL of **Detection Antibody** per well.
7. Seal **assay plate** and incubate for 0.5 hour at 25°C on microplate incubator/shaker.

0.5 hour 25°C

8. Perform Post-Detection Wash**.**
9. Perform Post-Detection Shake for 2 minutes on Jitterbug setting #3**.**
10. Perform Final Aspiration.
11. Remove from washer magnet and add 10 µL of **Elution Buffer B** to each well
12. Seal and incubate for 10 minutes at 25°C on microplate incubator/shaker.

10 minutes 25°C

13. Neutralize eluted antibody.
14. Seal **reading plate** with aluminum adhesive plate seal for SMCxPRO™.

SMCxPRO™ SYSTEM

ASSAY CHARACTERISTICS

A. Analysis

Raw Data should be exported to txt and analyzed in Excel. RE values can be compaired when the TP/Sec value is less than 800,000. Samples with values above 800,000 TP/Sec should be further diluted to align into the RE range, or they can be compaired using the TP/Sec values.

B. NIBSC Research Reagent for anti-SARS-CoV-2 Ab

NIBSC Code 20/130 was purchased as a solvent-detergent treated positive control and gave a positive result in our lab at up to 1,000 fold-dilution.

C. Matrix

Average %CV between matched Serum and EDTA Plasma was 4%

D. Sensitivity and Specificity

Two examples of cut-points generated based on data collected with this kit with Sars-cov-2 positive and known negative samples.

Cutoff	RE 400	RE 600
Sensitivity	0.8805	0.8491
Specificity	0.9809	0.9873
AUC	0.981	0.981

E. Precision

The assay variation of the SMC™ Human SARS-CoV-2 RBD IgG Immunoassay kit were studied using twenty-one plasma samples, with roughly half positive and half negative, run in triplicate by 2 different operators on 2 different days.

Mean intra-assay variation for samples above 400RE was [< 14%].

Mean intra-assay variation for samples below 400RE was [< 18%].

Mean intra-assay variation for all samples was [< 15%].

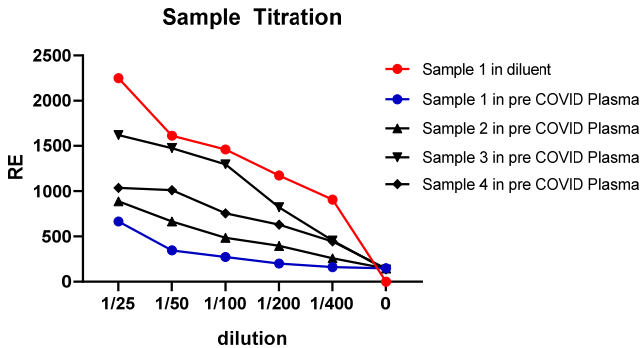
Mean inter-assay variation for samples above 400RE was [< 9%].

Mean inter-assay variation for samples below 400RE was [< 29%].

Mean inter-assay variation for all samples was [< 21%].

F. Sample titering on SMCxPRO™ instrument

SMCxPRO™ signal dilution was evaluated for one COVID-positive sample serially diluted in kit standard diluent, and four COVID-positive samples serially diluted in pre-COVID plasma. Sample dilutions demonstrate good correlation with instrument response for all cases.



TROUBLESHOOTING GUIDE

Problem	Probable Cause	Solution
Background is too high	Background wells were contaminated	Avoid cross-well contamination by using seal appropriately. Pipette with multichannel pipets without touching reagent in plate. Change tips when adding reagents if cross contamination is expected.
		Ensure reagents (including wash and system buffers) are not contaminated.
		Insufficient washes—washer may need to be cleaned or reprogrammed.
	Plate was over-incubated	Confirm plate incubation times are as recommended, particularly for the Detection incubation.
Sample variability is high	Multichannel pipet may not be calibrated	Calibrate pipets.
	Plate washing was not uniform	Confirm that there is no residual left in the wells following post-capture wash step and Final Aspirate. Ensure that you have < 2 µL or residual remaining in the well.
	Samples may have high particulate matter or other interfering substances	Samples should be centrifuged or filtered according to the Assay Preparation section. Unprocessed samples could lead to higher imprecision.
	Plate agitation was insufficient	Plate should be agitated during all incubation steps using a vertical plate shaker at a speed where beads are in constant motion without causing splashing (~650 - 1000 RPM).
	Cross-well contamination	Ensure that the plate is sealed well at each incubation step. If splashing occurs on plate seal, centrifuge plate at 1,100 x g for 1 minute to remove material prior to removing the seal. A new plate seal should be used every time the plate is sealed.
		Care should be taken when using same pipet tips that are used for reagent additions and that pipet tip does not touch reagent in plate.
Beads are lost during the wash	Plate washer needs optimization/cleaning	Contact Tech Support or local BCS to schedule washer programming. Refer to user guide for cleaning procedure.
	Insufficiently primed washer	Washer should be primed with wash buffer prior to running the post capture wash protocol.

TROUBLESHOOTING GUIDE (continued)

Problem	Probable Cause	Solution
Beads are lost during the wash (continued)	Beads came in contact with water	Washer should be primed with wash buffer sufficiently prior to plate wash. Viscosity of water changes the performance of the magnetic particles.
	Proper magnet was not used	Ensure that the mag plate (EMD Millipore PN 90-0003-02) was present on plate wash stage prior to running wash protocol.
Microparticles do not resuspend into homogenous solution	Beads were not properly stored and may have been frozen	Labelled microparticles should be stored at 4°C. If microparticles are frozen they will not resuspend properly.
	Samples may be causing interference due to excess particulate matter	Samples should be properly processed prior to testing to remove particulate matter or lipids.

ORDERING INFORMATION

To place an order or to obtain additional information about SMC™ products, please contact your Customer Service or Technical Support Specialist.

Contact information for each region can be found on our website:

emdmillipore.com/contact

Conditions of Sale

For Research Use Only. Not for Use in Diagnostic Procedures.

Safety Data Sheets (SDS)

Safety Data Sheets for EMD Millipore products may be ordered by fax or phone or through our website at emdmillipore.com/msds

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample1 1:50	Sample1 1:50	Sample1 1:50									
B	Sample1 1:100	Sample1 1:100	Sample1 1:100									
C	Sample1 1:200	Sample1 1:200	Sample1 1:200									
D	Sample1 1:400	Sample1 1:400	Sample1 1:400									
E	Sample1 1:800	Sample1 1:800	Sample1 1:800									
F	Sample1 1:1600	Sample1 1:1600	Sample1 1:1600									
G	Pre covid sample 1:50	Pre covid sample 1:50	Pre covid sample 1:50									
H	Diluent	Diluent	Diluent									

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