SARS-Cov2 anti-RBD IgG ELISA

96-Well Plate Assay

Cat. # EZRBDG-110K
EZRBDG-110K-5PBK

SARS-Cov2 anti-RBD IgG ELISA KIT
96-Well Plate

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INTENDED USE

This SARS-Cov2 anti-RBD IgG ELISA kit is used for the non-radioactive quantification of human SARS-Cov2 anti-RBD IgG antibodies in serum and plasma. One kit is sufficient to measure 38 unknown samples in duplicate. *This kit is for Research Use Only. Not for Use in Diagnostic Procedures.*

PRINCIPLES OF ASSAY

This assay is a Sandwich ELISA based, sequentially, on: 1) capture of human SARS-Cov2 anti-RBD IgG antibodies from samples to the wells of a microtiter plate coated with the SARS-CoV2 RBD antigen, 2) washing of unbound materials from samples, 3) binding of a streptavidin-horseradish peroxidase conjugated polyclonal goat anti-Human IgG antibody to the captured antibodies, 4) washing of unbound materials from samples, 5) quantification of immobilized antibody-enzyme conjugates by monitoring horseradish peroxidase activities in the presence of the substrate 3,3',5,5'-tetramethylbenzidine. The enzyme activity is measured spectrophotometrically by the increased absorbance at 450 nm – 590 nm after acidification of formed products. Since the increase in absorbance is directly proportional to the amount of captured human SARS-Cov2 anti-RBD IgG antibodies in the unknown sample, the latter can be derived by interpolation from a reference curve generated in the same assay with reference standards of known concentrations of monoclonal human SARS-Cov2 anti-RBD IgG.

REAGENTS SUPPLIED

Each kit is sufficient to run one 96-well plate and contains the following reagents:

Note: Store all reagents at 2-8°C

Reagents Supplied	Catalog Number	Volume	Quantity
Microtiter Plate with 2 plate sealers	EP-RBD	---------	1 plate 2 sealers
RBD Monoclonal IgG Antibody Standard	E8110-K	lyophilized	1 vial
RBD Monoclonal IgG Antibody Quality Controls 1 and 2	E6110-K	lyophilized	2 vials
Assay Buffer	AB-P	40 mL	1 bottle
10X Wash Buffer	EWB-HRP	50 mL	2 bottles
RBD IgG HRP-Detection Conjugate	E1110	12 mL	1 bottle
Substrate Solution	ESS-TMB3	12 mL	1 bottle
Stop Solution	ET-TMB	12 mL	1 bottle

STORAGE AND STABILITY

Recommended storage for kit components is 2-8°C.

All components are shipped and stored at 2-8°C. Reconstituted standards and controls can be frozen for future use, but repeated freeze/thaw cycles should be avoided. Refer to expiration dates on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot numbers.

REAGENT PRECAUTIONS

Sodium Azide or Proclin has been added to some reagents as a preservative. Although the concentrations are low, Sodium Azide and Proclin may react with lead and copper plumbing to form highly explosive metal azides. Dispose of unused contents and waste in accordance with international, federal, state, and local regulations.

Full Hazard Label

Ingredient, Cat #		Full Label	
RBD Monoclonal IgG Antibody Standard/RBD Monoclonal IgG Antibody Quality Control 1 & 2	E8110-K/E6110-K		**Danger.** Harmful if swallowed or if inhaled. Toxic in contact with skin. Causes serious eye damage. May cause damage to organs through prolonged or repeated exposure. May cause damage to organs Respiratory Trac or Brain through prolonged or repeated exposure if swallowed. Harmful to aquatic life with long lasting effects. Do not breathe dust/ fume/ gas/ mist/ vapours/ spray. Wash skin thoroughly after handling. Do not eat, drink or smoke when using this product. Use only outdoors or in a well-ventilated area. Avoid release to the environment. Wear protective gloves/ protective clothing/ eye protection/ face protection. IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth. IF ON SKIN: Wash with plenty of water. Call a POISON CENTER/ doctor if you feel unwell. IF INHALED: Remove person to fresh air and keep comfortable for breathing. Call a POISON CENTER/ doctor if you feel unwell. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Get medical advice/ attention if you feel unwell. Take off contaminated clothing and wash before reuse. Store locked up. Dispose of contents/ container to an approved waste disposal plant.

Full Hazard Label Continued

Ingredient, Cat #		Full Label	
RBD IgG HRP-Detection Conjugate	E1110		**Warning.** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.
10X HRP 10X Wash Buffer Concentrate	EWB-HRP		**Warning.** May cause an allergic skin reaction. Wear protective gloves. IF ON SKIN: Wash with plenty of soap and water.
Stop Solution	ET-TMB		**Warning.** May be corrosive to metals.

MATERIALS REQUIRED BUT NOT PROVIDED

1. Multi-channel Pipettes and pipette tips: 5-50 µL and 50-300 µL
2. Pipettes and pipette tips: 10 µL-20 µL or 20 µL-100 µL
3. Reagent Reservoirs
4. Polypropylene Microfuge Tubes
5. Vortex Mixer
6. De-ionized water
7. Microtiter Plate Reader capable of reading absorbency at 450 nm
8. Orbital Microtiter Plate Shaker
9. Absorbent Paper or Cloth

SAMPLE COLLECTION AND STORAGE

A. Preparation of Serum Samples:

- Allow the blood to clot for at least 30 minutes before centrifugation for 10 minutes at 1000xg. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple **>2** freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
- Serum sample dilution should be determined by the end user for SARS-CoV2 samples. This assay was tested at 1:100 with normal samples. For example, in a tube, **5 µL** of serum may be combined with **495 µL of Assay Buffer provided in the kit**. When further dilution beyond **1:100** is required, use **Assay Buffer** as the diluent.

B. Preparation of Plasma Samples:

- Plasma collection using EDTA as an anti-coagulant is recommended. Centrifuge for 10 minutes at 1000xg within 30 minutes of blood collection. Remove plasma and assay immediately or aliquot and store samples at ≤ -20°C.
- Avoid multiple **>2** freeze/thaw cycles.
- When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.
- Plasma sample dilution should be determined by the end user for SARS-CoV2 samples. This assay was tested at 1:100 with normal samples. For example, in a tube, **5 µL** of plasma may be combined with **495 µL of Assay Buffer provided in the kit**. When further dilution beyond **1:100** is required, use **Assay Buffer** as the diluent.

NOTE:

- A maximum of **100** µL per well of **diluted** serum or plasma can be used.
- All samples must be stored in polypropylene tubes. **DO NOT STORE SAMPLES IN GLASS.**
- Avoid debris, lipids and cells when using samples with gross hemolysis or lipemia.
- Care must be taken when using heparin as an anti-coagulant since an excess of heparin will provide falsely high values. Use no more than 10 IU heparin per mL of blood collected.

REAGENT PREPARATION

A. RBD Monoclonal IgG Antibody Standard Preparation

1. Use care in opening the lyophilized Standard vial. Using a pipette, reconstitute the RBD Monoclonal IgG Antibody Standard with 1.0 mL distilled or de-ionized water. Invert and mix gently, let sit for 5 minutes then mix well.

2. Label 6 polypropylene microfuge tubes as tubes 1, 2, 3, 4, 5 and 6. Add 0.23 mL of Assay Buffer to each of the 6 tubes. Prepare serial dilutions by adding 0.23 mL of the reconstituted standard to tube 6, mix well and transfer 0.23 mL of the 6 standard to tube 5, mix well and transfer 0.23 mL of the 5 standard to tube 4, mix well and transfer 0.23 mL of the 4 standard to tube 3, mix well and transfer 0.23 mL of the 3 standard to tube 2, mix well and transfer 0.23 mL of the 2 standard to tube 1 and mix well. The 0 ng/mL standard (Background) will be Assay Buffer.

   Note: Change tip for every dilution. Wet tip with standard before dispensing. Unused portions of reconstituted standard should be stored in small aliquots at ≤ -20°C. Avoid multiple freeze/thaw cycles.

Tube #	Volume of Deionized Water to Add	Volume of Standard to Add	Standard Stock Concentration
Reconstituted standard	1 mL	0	X (refer to analysis sheet for exact concentration)

Tube #	Volume of Assay Buffer to Add	Volume of Standard to Add	Standard Concentration (ng/mL)
6	0.23 mL	0.23 mL of reconstituted standard	X/2
5	0.23 mL	0.23 mL of Tube 6	X/4
4	0.23 mL	0.23 mL of Tube 5	X/8
3	0.23 mL	0.23 mL of Tube 4	X/16
2	0.23 mL	0.23 mL of Tube 3	X/32
1	0.23 mL	0.23 mL of Tube 2	X/64

REAGENT PREPARATION (continued)

B. RBD Monoclonal IgG Antibody Quality Control 1 and 2 Preparation
Use care in opening the lyophilized Quality Control vials. Reconstitute each RBD Monoclonal IgG Antibody Quality Control 1 and Quality Control 2 with 1.0 mL distilled or de-ionized water and gently invert to ensure complete hydration. Unused portions of the reconstituted Quality Controls should be stored in small aliquots at ≤ -20°C. Avoid further freeze/thaw cycles.

C. Preparation of Wash Buffer

Bring the 10X Wash Buffer to room temperature and mix to bring all salts into solution. Dilute 100 mL of 10X Wash Buffer (two bottles) with 900 mL deionized water. Store unused portion at 2-8°C for up to one month.

SARS-Cov2 anti-RBD IgG ELISA ASSAY PROCEDURE

Warm all reagents to room temperature before setting up the assay.

1. Remove the required number of strips from the Microtiter Assay Plate. Unused strips should be resealed in the foil pouch and stored at 2-8°C. Assemble the strips in an empty plate holder. Add 300 µL diluted Wash Buffer to each well of the plate. Decant Wash Buffer and remove the residual volume by inverting the plate and tapping it smartly onto absorbent towels several times. Repeat wash procedure 2 additional times. Do not let wells dry before proceeding to the next step. If an automated machine is used for the assay, follow the manufacturer's instructions for all washing steps described in this protocol.

2. Add 100 µL Assay Buffer to each of the Blank wells.

3. Add 100 µL Standards or Controls to the appropriate wells.

4. Add 100 µL of diluted sample to the appropriate wells.

5. Cover the plate with plate sealer and incubate at room temperature for 1 hour on an orbital microtiter plate shaker set to rotate at moderate speed, about 400 to 500 rpm.

6. Remove plate sealer and decant reagents from the plate. Tap as before to remove residual volume in well. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

7. Add 100 µL HRP-Detection Conjugate to each well. Re-cover plate with sealer and incubate at room temperature for 1 hour on an orbital microtiter plate shaker set to rotate at moderate speed, approximately 400-500 rpm.

8. Remove plate sealer and decant reagents from the plate. Tap as before to remove residual volume in well. Wash wells 3 times with diluted Wash Buffer, 300 µL per well per wash. Decant and tap after each wash to remove residual buffer.

9. Add 100 µL of Substrate Solution to each well, cover plate with sealer and shake on the plate shaker for approximately 5 to 20 minutes. Blue color should be formed in wells of the RBD Monoclonal IgG Antibody standards with intensity proportional to increasing concentrations of RBD Monoclonal IgG Antibody.

   Note: Please be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Please visually monitor the color development to optimize the incubation time.

10. Remove sealer and add 100 µL Stop Solution [CAUTION: CORROSIVE SOLUTION] and gently shake plate by hand to ensure complete mixing of solution in all wells. The blue color should turn to yellow after acidification. Wipe the bottom of the microtiter plate to remove any residue prior to reading on plate reader. Read absorbance at 450 nm and 590 nm in a plate reader within 5 minutes and ensure that there are no air bubbles in any well. Record the difference of absorbance units. The absorbance of the highest RBD Monoclonal IgG Antibody standard should be approximately 2.0 - 3.0, or not to exceed the capability of the plate reader used.

    Note: Samples are diluted 1:100 or appropriate dilution determined by the end user. Final results should be multiplied by a dilution factor of 100 or factor determined by the end user.

Assay Procedure for SARS-Cov2 anti-RBD IgG ELISA Kit (Cat. # EZRBDG-110K)

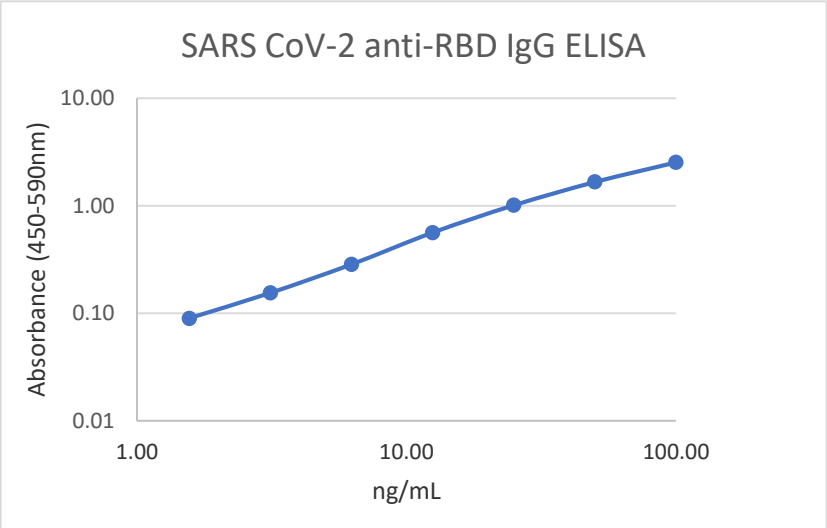
	Step 1	Step 2	Step 3-4	Step 5-6	Step 7	Step 8	Step 9-10			
Well #	**Wash plate 3X with 300 µL 1X Wash Buffer. Remove residual buffer by tapping smartly on absorbent towels.**	Assay Buffer	Standards/ QCs/ Samples	**Seal, Agitate, Incubate 1 hr at Room Temperature. Wash 3X with 300 µL Wash Buffer.**	Detection Conjugate	**Seal, Agitate, Incubate 1 hour at Room Temperature. Wash 3X with 300 µL Wash Buffer.**	Substrate	**Seal, Agitate, Incubate 5 – 20 minutes at Room Temperature.**	Stop	**Read Absorbance at 450 nm and 590 nm.**
A1, B1		**100** µL	--		**100** µL		**100** µL		**100** µL	
C1, D1		--	100 µL of Tube 1							
E1, F1		--	100 µL of Tube 2							
G1, H1		--	100 µL of Tube 3							
A2, B2		--	100 µL of Tube 4							
C2, D2		--	100 µL of Tube 5							
E2, F2		--	100 µL of Tube 6							
G2, H2		--	100 µL of Reconstituted Standard							
A3, B3		--	100 µL of QC1							
C3, D3			100 µL of QC2							
E3, F3			100 µL of Diluted Sample							
G3, H3 Etc.			100 µL of Diluted Sample							

MICROTITER PLATE ARRANGEMENT

SARS-Cov2 anti-RBD IgG ELISA

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Tube 4	QC1									
B	Blank	Tube 4	QC1									
C	Tube 1	Tube 5	QC2									
D	Tube 1	Tube 5	QC2									
E	Tube 2	Tube 6	Diluted Sample 1									
F	Tube 2	Tube 6	Diluted Sample 1									
G	Tube 3	Reconstituted Standard	Diluted Sample 2									
H	Tube 3	Reconstituted Standard	Diluted Sample 2									

GRAPH OF TYPICAL REFERENCE CURVE



Typical Standard Curve, not to be used to calculate data.

ASSAY CHARACTERISTICS

A. Sensitivity

The lower limit of quantitation (LLOQ) of human SARS CoV-2 anti-RBD IgG assay is 1.56 ng/mL using Belysa™ Immunoassay Analysis software from Millipore Sigma. LLOQ is calculated by back interpolation of the standard point that provides CV≤ 20% and recovery ± 20% of the expected.

ASSAY CHARACTERISTICS (continued)

B. Precision

Intra-Assay Variation

	Mean anti-RBD IgG Levels (ng/mL)	Intra-Assay %CV
1	8.5	≤ 5%
2	27.4	≤ 5%

Inter-Assay Variation

	Mean anti-RBD IgG Levels (ng/mL)	Inter-Assay %CV
1	8.2	≤ 10%
2	25.7	≤ 10%

The assay variations of EMD Millipore's human SARS-Cov2 anti-RBD IgG ELISA kit was studied on two samples at two levels on the human SARS-Cov2 anti-RBD IgG standard curve. The mean intra-assay variation was calculated from results of eight determinations of the indicated samples. The mean inter-assay variations of each sample were calculated from results of 8 separate assays with duplicate samples in each assay.

ASSAY CHARACTERISTICS (continued)

C. Spike Recovery of human SARS-Cov2 anti-RBD IgG in Assay Samples

Sample	anti-RBD IgG Added (ng/mL)	Expected (ng/mL)	Observed (ng/mL)	Recovery
1	6.25	15.1	15.0	99%
	12.5	21.4	22.4	105%
	25	33.9	37.6	111%
2	6.25	7.36	6.7	91%
	12.5	13.6	13.1	96%
	25	26.1	27.3	104%
3	6.25	7.07	6.4	91%
	12.5	13.3	12.2	91%
	25	25.8	25.9	100%
4	6.25	6.77	6.1	91%
	12.5	13.0	12.4	96%
	25	25.5	25.8	101%
5	6.25	7.11	6.6	93%
	12.5	13.4	12.7	95%
	25	25.9	28.1	109%
Average				98%

Varying amounts of SARS-Human Cov2 anti-RBD IgG monoclonal antibody were added to individual human serum and plasma samples and the resulting SARS-Cov2 anti-RBD IgG content of each sample was assayed by human SARS-Cov2 anti-RBD IgG ELISA. The recovery = [(observed SARS-Cov2 anti-RBD IgG / (spiked SARS-Cov2 anti-RBD IgG concentration + basal SARS-Cov2 anti-RBD IgG level)] x 100%.

ASSAY CHARACTERISTICS (continued)

D. Linearity of Sample Dilution

Sample	Volume (µL)	Expected (ng/mL)	Observed (ng/mL)	Expected
1	100	29.1	29.1	
	50	14.6	14.2	98%
	25	7.3	6.7	92%
	12.5	3.6	3.3	90%
2	100	38.2	38.2	
	50	19.1	17.8	93%
	25	9.6	8.6	90%
	12.5	4.8	4.2	87%
3	100	28.1	28.1	
	50	14.0	13.5	96%
	25	7.0	6.4	92%
	12.5	3.5	3.2	92%
4	100	27.2	27.2	
	50	13.6	12.6	93%
	25	6.8	5.9	87%
	12.5	3.4	3.0	90%
5	100	27.6	27.6	
	50	13.8	13.0	94%
	25	6.9	6.4	93%
	12.5	3.5	3.2	94%
Average				92%

Five human serum and plasma samples spiked with SARS CoV-2 anti-Human RBD IgM monoclonal antibody with the indicated sample volumes were assayed. Required amounts of assay buffer were added to compensate for lost volumes below 100 µL. The resulting dilution factors of neat, 2, 4 and 8 representing 100 µL, 50 µL, 25 µL and 12.5 µL sample volumes assayed, respectively, were applied in the calculation of observed human SARS-Cov2 anti-RBD IgG concentrations. % expected = (observed/expected) x 100%

QUALITY CONTROLS

The ranges for Quality Control 1 and 2 are provided on the card insert or can be located at the EMD MILLIPORE website www.emdmillipore.com.

TROUBLESHOOTING GUIDE

1. To obtain reliable and reproducible results the operator should carefully read this manual and fully understand all aspects of each assay step before attempting to run the assay.
2. Throughout the assay the operator should adhere strictly to the procedures with good laboratory practice.
3. Have all necessary reagents and equipment ready on hand before starting. Once the assay has been started all steps should be completed with precise timing and without interruption.
4. Avoid cross contamination of any reagents or samples to be used in the assay.
5. Make sure all reagents and samples are added to the bottom of each well.
6. Careful and complete mixing of solutions in the well is critical. Poor assay precision will result from incomplete mixing or cross well contamination due to inappropriate mixing.
7. Remove any air bubbles formed in the well after acidification of substrate solution because bubbles interfere with spectrophotometric readings.
8. High signal in background or blank wells could be due to 1.) cross well contamination by standard solution or sample or 2.) inadequate washing of wells with Wash Buffer or 3.) overexposure to light after substrate has been added.

REPLACEMENT REAGENTS

Reagents	Cat. #
Human RBD ELISA Plate	EP-RBD
10X HRP Wash Buffer Concentrate	EWB-HRP
RBD Monoclonal IgG Antibody ELISA Standard	E8110-K
RBD Monoclonal IgG Antibody Quality Controls 1 and 2	E6110-K
Assay Buffer	AB-P
RBD IgG ELISA HRP-Detection Antibody Conjugate	E1110
Substrate Solution	ESS-TMB3
Stop Solution	ET-TMB
SARS-Cov2 anti-RBD IgG ELISA (5 pk bulk)	EZRBDG-110K-5PBK

ORDERING INFORMATION

To place an order or to obtain additional information about our immunoassay 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.