READING RESULTS

A. TEST VALIDATION

The test **is** valid if the mean OD_{450} of the Positive Control is **> 0.5** and the ratio (mean OD_{450} Positive Control / mean OD_{450} Negative Control) is **> 5.0**.

B. INTERPRETATION OF THE TEST

The **IRPC** value (Relative Index x 100) must be obtained for each sample when interpreting the results. The following ratio must be applied to obtain the IRPC value for each sample (The mean OD_{450} values obtained with 2 control replicates are used):

$$IRPC = \left[\frac{OD_{450}\ Sample - Mean\ OD_{450}\ Negative\ Control}{Mean\ OD_{450}\ Positive\ Control - Mean\ OD_{450}\ Negative\ Control} \right] \times 100$$

IRPC VALUE	Immune status against *SARS-CoV-2*
Lower than 20.0 or equal	NEGATIVE – undetectable IgG antibodies against SARS-CoV-2
Higher to 20.0	POSITIVE – presence of IgG antibodies against SARS-CoV-2

TEST DEVELOPMENT

1.	Control and Samples	
2.	Incubate	60 min · +36 °C - +38 °C
3.	Wash (3 times)	
4.	Conjugate Solution	
5.	Incubate	60 min · +36 °C - +38 °C
6.	Wash (3 times)	
7.	Substrate Solution	
8.	Incubate in the dark	15 min · +20 - +25 °C (Room Temp.)
9.	Stop Solution	
10.	Results Reading (450 nm)	

Manufactured by:
LABORATORIOS HIPRA, S.A.
Avda. la Selva, 135 . 17170 Amer (Girona) SPAIN
Tel. (34) 972 43 06 60. Fax (34) 972 43 06 61. hipra@hipra.com

729656-20.1

06-20

EN

HIPRACHECK SARS-CoV-2 IgG

Detection and quantification of specific IgG antibodies,
against SARS-CoV-2 in human serum or plasma,
by indirect ELISA

CE IVD

TEST PRINCIPLE

The Coronavirus 2 (CoV-2) responsible for Severe Acute Respiratory Syndrome (SARS-CoV-2) is an enveloped, non-segmented RNA virus with positive polarity. It is the cause of the so-called Coronavirus Disease of 2019 (COVID-19). There are two main types of tests for laboratory diagnosis of COVID-19: (i) SARS-CoV-2 viral RNA screening tests (primarily using qPCR) that identify individuals infected with the virus during the acute phase of infection and (ii) serological tests that identify individuals who have been in contact with the virus and have developed a specific humoral response against it.

HIPRACHECK SARS-CoV-2 **IgG** is a test based on an indirect enzyme immunoassay (ELISA), designed for serological analysis of **human serum or plasma samples** (for both venous and capillary extraction). This test enables the detection of IgGs specifically directed against SARS-CoV-2. Some of the main antigenic determinants of the virus, recombinantly expressed and highly purified, are used to cover the 96 wells of each microplate. In this manner, the SARS-CoV-2 specific IgGs present in the samples can form antigen-antibody complexes (Ag-Ab) with the protein immobilized in the well, and thus are retained therein. The presence of these Ag-Ab complexes is detected due to an anti-human IgG antiserum conjugated to peroxidase. The use of a chromogenic substrate specific for this enzyme (TMB) reveals the presence of the Ag-Ab complexes retained in the well. Hence, the consequent appearance of colour in each well is proportional to the amount of SARS-CoV-2 specific IgGs present in the initial sample.

KIT COMPOSITION (ENOUGH FOR A MAXIMUM OF 184 TESTS)

PRODUCT	QUANTITY
96-well microplates (divided into eight-well strips) coated with the specific SARS-CoV-2 antigen.	**2**
Vial N°0: Washing solution (10x).	**60 ml**
Vial N°1: Sample Diluent (3x): Concentrated sample diluent solution with green dye.	**60 ml**
Vial N°2: Conjugate Solution: Anti-IgG human/HRPO solution containing red dye. Ready to use.	**15 ml**
Vial N°3: Substrate solution: TMB solution. Ready to use.	**15 ml**
Vial N°4: Stop Solution: H_2SO_4 solution. Ready to use.	**15 ml**
Vial N°5: Positive Control: Positive solution of monoclonal specific IgG antibody against SARS-CoV-2, containing yellow dye. Ready to use.	**1.0 ml**
Vial N°6: Negative Control: Negative solution of monoclonal specific IgG antibody against SARS-CoV-2, containing blue dye. Ready to use.	**1.0 ml**
Microplate adhesive cover.	**2**
Kit insert.	**1**

Material required but not provided:
+37 °C incubator, precision pipettes (single or multichannel) with their corresponding tips, tubes and U-bottom plates to dilute the samples, ELISA plate reader, distilled or deionised water and plate washer.

PRECAUTIONS

Carefully read this kit insert. Store all reagents between +2 and +8 °C (do not freeze). Store the unused plates between +2 and +8 °C duly sealed with the adhesive cover inside the plastic bag with silica gel. Do not expose substrate solution to strong light or any oxidising agents. ***The TMB is very sensitive to any mixture or contamination; therefore, it should not be returned to the bottle once removed. A 25% excess of this reagent is provided to allow a small excess to be taken each time it is used.*** Do not use the kits after expiry date and do not intermix reagents or instructions from kits with different lot numbers. Careful pipetting and washing throughout the procedure are necessary to maintain precision. Do not pipette the reagents by mouth. Wear gloves during the process. The stop solution contains 2N sulphuric acid (corrosive), handle with care. All waste materials must be properly decontaminated prior to disposal. **Unopened reagents, correctly stored, are stable until the expiry date printed in the external label.**

TEST PROCEDURE

A. REAGENT PREPARATION

Let the reagents reach room temperature before beginning the assay procedure.

Washing Solution (10x) (Vial N°0): To reconstitute, add 1 volume of Washing Solution (10x) to 9 volumes of **distilled or deionised** water (e.g. to prepare 200 ml of diluted Washing Solution, mix 20 ml of the concentrate solution (10x) with 180 ml of **distilled or deionised water**). The diluted solution is stable up to 7 days.

Sample Dilution (3x) (Vial N°1): To reconstitute it add 1 volume of Sample Dilution (3x) to 2 volumes of **distilled or deionised water** (e.g. to prepare 60 ml of diluted Sample Dilution, mix 20 ml of the concentrate solution (3x) with 40 ml of **distilled or deionised water**). The diluted solution is stable up to 7 days.

NOTE: In its concentrated form, it is possible that after prolonged periods of storage at +4 °C, crystals will form in both the Wash Solution (10x) and Sample Diluent Solution (3x). If you intend to reconstitute the entire volume supplied at once, simply shaking the inverted bottle several times is enough to reconstitute it. If only part of the volume is reconstituted (the volume to be used for the assay), make sure that the crystals are completely re-dissolved before preparing the dilution. To speed up the process, the bottle can be immersed in a bath at +28 - +37 °C for 10 - 15 min. To avoid the appearance of crystals in the concentrated washing solution, it can be stored at room temperature throughout the life of the kit.

B. SAMPLE PREPARATION

The positive and negative controls are ready for use and do not require dilution. The rest of the samples must be diluted **1/100** in diluted Sample Diluent Solution. For biosafety reasons, it is recommended to previously inactivate the samples by means of thermal treatment at +56 °C for 30 minutes. This treatment is described as inactivating SARS-CoV-2.

If you have 96-well serological plates with a U-bottom and a multi-channel pipette capable of accurately dispensing volumes of 10 µl, it is recommended to **perform the 1/100 dilution in two steps** as follows: Dilute initially, in the serology plate, 10 µl of sample in 190 µl of diluted Sample Diluent Solution, and then transfer 10µl of this 1/20 dilution of the sample to a well of the ELISA plate already containing 40 µl of diluted Sample Diluent Solution.

C. TEST DEVELOPMENT

A. Allow the reagents to come to room temperature and ensure adequate mixing by swirling or inversion.

B. Prepare a data sheet to identify the individual wells for each sample and control. The positive and negative controls must always be run in duplicate.

1. Peel off the plastic adhesive cover and add **50 µl of undiluted controls and 50 µl of diluted samples (1/100)** to the appropriate wells in the plate.
2. Cover the plate with the adhesive cover and incubate **60 minutes at +36 °C - +38 °C**.
3. Remove the adhesive cover and wash each well **3 times** with 300 µl of diluted Washing Solution. Invert and firmly tap the plate on absorbent paper.
4. Add **50 µl of Conjugate Solution** (Vial N°2) to each well.
5. Cover the plate with the adhesive cover and incubate **60 minutes at +36 °C - +38 °C**.
6. Remove the adhesive cover and wash each well **3 times** with 300 µl of diluted Washing Solution. Invert and firmly tap the plate on absorbent paper.
7. Add **50 µl of Substrate Solution** (Vial N°3) to each well. Shake the plate gently for 2 seconds.
8. Seal the plate with an adhesive cover and incubate at **room temperature (+20 °C - +25 °C)** in the dark **for 15 minutes**.
9. Remove the adhesive cover and dispense **50 µl of Stop Solution** (Vial N°4) into each well. Shake by lightly tapping the side of the microplate.
10. Clean the bottom surface of the plate with an absorbent paper. **Read** the plate using an ELISA Reader equipped with a **450 nm** filter. Make the blank on air ahead of time. Record the results.