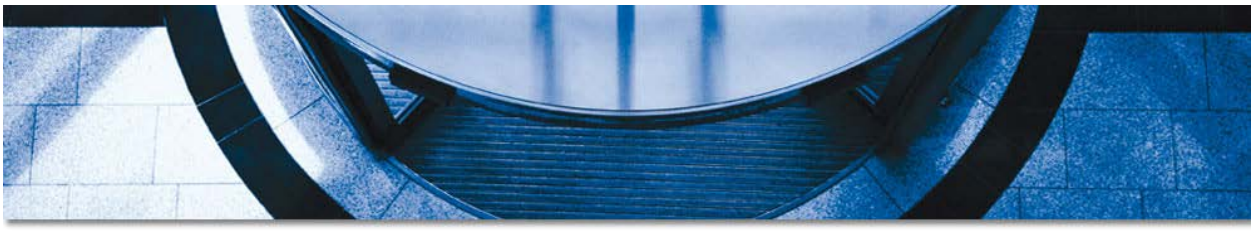
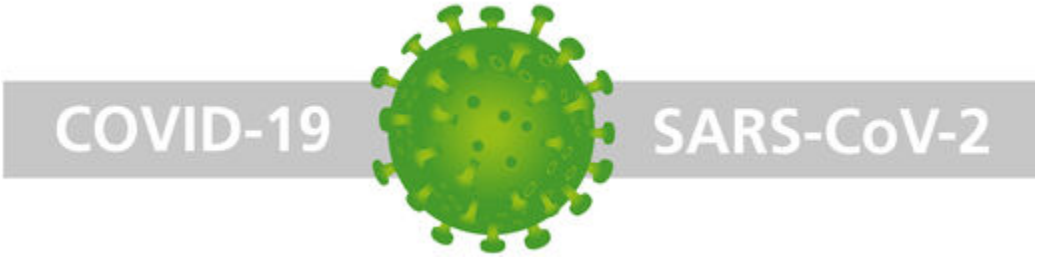
MIKROGEN
DIAGNOSTIK





Product No. 7304

*recom*Well SARS-CoV-2 IgG

1 Purpose

For sale for Research Use Only. Not for diagnostic use.

2 Intended Use

MIKROGEN's *recom*Well SARS-Cov-2 IgG detects IgG antibodies against SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2). **This test must not be used for clinical diagnosis!**

3 Test Principle

Highly purified recombinant nucleocapsid protein of SARS-CoV-2 is fixed to the wells of a microplate.

1. Diluted serum or plasma samples are incubated in the wells; antibodies in the samples bind specifically to the antigen on the wells.
2. Unbound antibodies are washed away.
3. In a second step, anti-Human Immunoglobulin antibody, coupled to horseradish peroxidase (HRP) is incubated in the wells.
4. Unbound conjugate is washed away.
5. Specifically-bound antibodies are detected by a peroxidase-catalyzed color reaction. Where an antigen-antibody reaction has taken place, the color development of the chromogenic substrate is proportional to the quantity of bound SARS-CoV-2 IgG antibodies. The concentration of color development can be measured using a photometer so as to establish the concentration of anti-SARS-CoV-2 IgG antibodies in the samples.

4 Reagents

4.1 Package contents

The reagents in one package are sufficient for 96 tests. Each test kit contains:

MTP	**12 x 8** wells microplate coated with recombinant SARS-CoV-2 antigens in a vacuum pressure-sealed bag
WASHBUF 10X	**100ml** Wash buffer (ten times concentration) Contains phosphate buffer, NaCl and detergent Preservative: MIT (0.01%) and Oxypyrion (0.1%)
DILUBUF	**125ml** Dilution buffer (ready-to-use) Contains protein, detergent and blue dye Preservative: MIT (0.01%) and Oxypyrion (0.1%)
SUBS-TMB	**12ml** Chromogenic substrate tetramethylbenzidine (TMB, ready-to-use)
SOLN-STOP	**12ml** stop solution 24.9% phosphoric acid (H_3PO_4)(ready-to-use)
CONTROL + IgG	**450ul** positive control (**violet** cap), contains MIT (0.1%) and Oxypyrion (0.1%)
CONTROL ± IgG	**450ul** cutoff control (**yellow** cap), contains MIT (0.1%) and Oxypyrion (0.1%)
CONTROL – IgG	**450ul** negative control (**white** cap), contains MIT (0.1%) and Oxypyrion (0.1%)
CONJ-IgG	**500ul** anti-human IgG conjugate (**101-times concentrated**, cap) contains NaN3 (<0.1%, MIT (<0.01% and chlorazetamide (<0.1%)
INSTRU	**1** Instructions for use
EVALFORM	**1** Evaluation form
TAPE	**2** pieces of covering film

4.2 Materials required but not supplied

- Deionized water (high quality)
- Test tubes
- Vortex mixer or other rotators
- 8-channel pipette or washer with pump
- Clean measuring cylinders, 50ml and 1000ml
- Micropipettes with disposable tips, 10ul and 1000ul
- 10ml pipette or dispenser
- Incubation chamber 37°C
- Microplate photometer
- Timer
- Disposable protective gloves
- Waste container for biohazardous materials

5 Shelf life and handling

- Store reagents at +2°C to +8°C before and after use, **do not freeze**.
- Subject all ingredients for at least 30 minutes to room temperature (+18°C to +25°C) before beginning the test.
- The components dilution buffer, wash buffer, substrate, and stop solution for the *recom*Well test can be used across the whole range of parameters and batches. At the same time, the shelf life of these components is to be noted.
- The control sera and conjugates are batch-dependent and may not be used across the whole range of parameters or batches.
- Mix the concentrated conjugates, control reagents and samples well before use. Avoid build-up of foam.
- All MIKROGEN microplates are equipped with break-apart bars.
- The covering films are intended for single use only.
- All package are marked with an expiration date after which quality can no longer be guaranteed.
- Protect kit components from direct sunlight throughout the entire test procedure. The substrate solution (TMB) is especially sensitive to light.
- The test should only be carried out by trained and authorized personnel.
- In case of significant changes to the product or the regulations for use by the user, the application may lie outside the purpose indicated by MIKROGEN.
- Cross-contamination of samples or conjugates can lead to inaccurate test results. Add the samples and conjugate solution carefully. Make sure that incubation solutions do not flow over into other wells.
- Automation is possible; further information can be obtained from MIKROGEN.

6 Warnings and Precautions

- **FOR RESEARCH USE ONLY!**
- All blood products must be treated as potentially infectious.
- The microplate wells have been coated with inactivated whole cell lysates, bacterial, or viral antigens.
- After the addition of samples, the microplate wells must be considered potentially infectious and treated accordingly.
- For the production of control reagents, blood from donors is used which does not contain antibodies to HIV-1, HIV-2, HCV, and HBs antigen. These reagents must be treated with the same care as experimental samples.
- Suitable disposable gloves must be worn throughout the entire test procedure.
- The conjugates contain the antimicrobial agents and preservatives sodium azide, MIT (methylisothiazolone), oxypyrion, chloroazetamide and hydrogen peroxide. Avoid contact with skin or mucous membranes. Sodium azide can form an explosive azide upon contact with heavy metals such as copper and lead.
- Phosphoric acid is an irritant. It is mandatory to avoid contact with skin and mucous membranes.
- All fluids to be disposed must be collected. All collection containers must contain suitable disinfectants for the inactivation of human pathogens. All reagents and materials contaminated with potentially infectious samples must be treated with disinfectants or disposed of according to

the hygiene regulations. The concentrations and incubation periods stated by the manufacturer must be observed.

- Only use microplate wells once.
- Do not substitute or mix the reagents with reagents from other manufacturers.
- Read through the entire instructions for use before carrying out the test and carefully follow them. Deviation from the test protocol provided in the instructions for use can lead to erroneous results.

7 Sampling and Preparation

7.1 Samples

The samples can be serum or plasma (EDTA, citrate, heparin, CPD) and must be separated from blood clots as soon as possible after blood collection so as to avoid hemolysis. Avoid microbial contamination of the samples. Insoluble substances must be removed from the samples prior to incubation. The use of heat-inactivated, icteric, hemolytic, lipemic, or turbid samples is not recommended.

CAUTION! If the tests are not carried out immediately, the samples can be stored for up to 2 weeks at +2°C to +8°C. Prolonged storage of the samples is possible at -20°C or less. Repeated freezing and thawing of samples is not recommended due to the risk of producing inaccurate results. Avoid more than 3 cycles of freezing and thawing.

7.2 Preparation of solutions

Contains sufficient reagents for 96 tests. The following quantity specifications relate to the processing of a single microplate strip with 8 wells. While using several microplate strips, the specified quantities must be simultaneously multiplied with the number of used microplate strips. Device-specific dead volume must be considered. Dilution buffer, substrate, and stop solution are ready-to-use.

7.2.1 *Preparation of ready-to-use wash buffer*

The wash buffer concentrate is diluted **1 + 9** with H_2O (deionized water). 5ml concentrate is mixed with 45ml H_2O (deionized water) per microplate strip with 8 wells. The ready-to-use wash buffer can be stored for 4 weeks at +2°C to +8°C or a week at room temperature.

7.2.2 *Preparation of conjugate solution*

For each microplate strip with 8 wells, 1ml of dilution buffer and 10ul of anti-human IgG-peroxidase conjugate (red cap) are transferred to a clean container and mixed well (dilution = **1+100**). The conjugate solution must be prepared <u>just before use</u>. It is not possible to store the ready-to use conjugate solution.

8 Test procedure

No.	Execution	Note
1	Expose all reagents for at least 30 minutes to +18°C to +25°C (room temperature) before beginning the test.	Bring the microplate to room temperature in **a sealed bag** to avoid condensation of water. Following the removal of the required strips, the plate must be resealed in the bag and stored in the refrigerator. Before use, thoroughly mix the control samples and experimental samples, as well as the concentrated conjugates, and then centrifuge briefly, where possible, in order to collect the fluid at the bottom of the containers.
2	Preparing samples and controls Pipette **10ul** of sample and/or control to every **1ml** dilution buffer and mix well (dilution = 1:100)	**Samples and controls must always be diluted immediately prior to carrying out the test.** For each test step, all of the controls must be carried out, diluted, just like the experimental samples.
3	Incubation of samples Pipette **100ul** of diluted sample and/or diluted control into each well and incubate for **1 hour at +37°C.**	Assign at least one well to the negative control, positive control, and experimental samples. The cutoff (±) control must be assigned to two wells. Preferably, a cutoff control should be included at the beginning of the series and at the end of the series. In manual processing, carefully cover tightly the microplate with unused cover film. Use the incubation chamber at +37°C.
4	Washing	It is recommended to carry out this step with a corresponding ELISA wash device. It is mandatory to ensure that the wash buffer is completely removed between the washing steps.
a) **b)** **c)**	Carefully remove the covering film. Completely empty the wells. Fill each of the wells with 300ul of ready-to-use wash buffer → (8.4b)	 Aspirate or pour out and beat out the contents. Carry out the washing steps 8.4b and 8.4c **four times** in total.
5	Incubation with conjugate Add 100ul of diluted conjugate solution (7.2.2) and incubate for **30 minutes at +37°C.**	In manual processing, the microplate is carefully covered tightly with unused cover film.
6	Washing (see 8.4b and 8.4c)	Carry out the washing steps four times in total.
7	Substrate reaction Pipette **100ul** of ready-to-use substrate solution into each well and incubate for **30 minutes at room temperature**. The time is calculated from pipetting into the first well.	It is not required to tightly cover the plate. Protect against direct exposure to sunlight.
8	Stopping the reaction Pipette **100ul** of ready-to-use stop solution into each well.	The substrate solution is not to be removed before adding the stop solution! The same pipetting scheme is to be followed as for the substrate solution.
9	Measurement of the absorbance values The absorbance values of the single wells are measured in a microplate photometer at 450nm and the reference wavelength 650nm (620 to 650nm permitted).	Zero adjustment is done against air. The measurement must be made within 60 minutes of stopping the reaction.
Caution! **Incubation solutions may not flow into other wells. Splashing must be avoided especially when removing and placing the cover film.**		

9 Results

9.1 Evaluation

Cutoff (limit) = the arithmetic mean is calculated from the absorbance values of both cutoff controls (at the beginning and at the end of the series).

9.1.1 *Qualitative evaluation*

Grey zone	Low limit = cutoff Upper limit = cutoff + 20% (cutoff x 1.2)
Negative	Samples with absorbance values **below** the grey zone
Borderline	Samples with absorbance values **within** the grey zone
Positive	Samples with absorbance values **above** the grey zone

9.1.2 *Quantitative evaluation*

The corresponding antibody activity in units per ml is assigned to the absorbance values using a formula. The measurement units U/ml are arbitrary units which do not allow conclusions concerning (international) reference values.

U/ml sample	(Absorbance sample / absorbance cutoff) x 20
Grey zone	Low limit = 20 U/ml Upper limit = 24 U/ml
Negative	U/ml sample <20
Borderline	20 ≤ U/ml sample ≤ 24
Positive	U/ml sample ≥ 24

9.2 Validation – Quality Control

The test can be evaluated under the following conditions:

- The single absorbance values of the double analysis of the cutoff control do not deviate by more than 20% from their average.
- Absorbance value – negative control ≤ 0.150.
- Cutoff control absorbance value – Negative control absorbance value ≥ 0.050.
- Positive control absorbance value – Cutoff control absorbance value ≥ 0.100.

These checks are used to validate the test results as per the “Validation Quality Control” chapter. The reproducibility of results can be improved by determining the specific antibodies relative to the cutoff check in U/ml as the fluctuations from the performance of the test are also included. In validating the test, the positive and negative checks do not need to be evaluated. If necessary, however, they can be carried out for internal quality control purposes. In this case, the results should lie within the target value range given in the analysis certificate or on the label.

10 Literature

1. S. Kannan, P. Shaik Syed Ali, A. Sheeza, K. Hemalatha. COVID-19 (Novel Coronavirus 2019) – recent trends. Eu. Rev. Med. Pharmacol. Sci. 2020;24:2006-2011

2. F. Amanat, T. H.O. Nguyen, V. Chromikova, S. Strohmeier, D. Stadlbauer, A. Javier, K. Jiang, G. A. Arunkumar, J. Polanco, M. Bermudez-Gonzales, D. Cap-livski, A. Cheng, K. Kedzierska, O. Vapalahti, J. M. Hepojoki, V. Simon, F. Krammer. A serological assay to detect SARS-CoV-2 seroconversion in humans. Preprint

3. N. M.A. Okba, M. A. Müller, W. Li, C. Wang, C. H. GeurtsvanKessel, V. M. Corman, M. M. Lamers, R. S. Sikkema, E. de Bruin, F. D. Chandler, Y.

11 Explanation of symbols

	Content is sufficient for <n> applications Number of applications
WASHBUF 10 X	Wash Buffer (ten times concentration)
DILUBUF	Dilution Buffer
SUBS TMB	Chromogenic substrate tetramethylbenzidine
SOLN STOP	Stop solution
TAPE	Covering films
MTP	Microtitre plate
CONTROL + IgG	Positive control IgG
CONTROL ± IgG	Cutoff control IgG
CONTROL - IgG	Negative control IgG
CONJ IgG	Anti-human IgG conjugate
TVALUE	Target and / or target range in U/ml
EVALFORM	Evaluation form
INSTRU	Instructions for use
	See instructions for use
CONT	Contents, includes
RUO	Research use only
LOT	Batch number
	Do not freeze
REF	Order number
	Use by Expiry date
	Store at x°C to y°C
	Manufacturer

12 Manufacturer and version information

***recom*Well SARS-CoV-2 IgG**		Item No. **7304**
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