*recom*Well SARS-CoV-2 IgG
*recom*Well SARS-CoV-2 IgA

MIKROGEN DIAGNOSTIK

IVD

Instructions for use (English)

1 Purpose

The *recom*Well SARS-CoV-2 IgG, IgA is a qualitative and/or quantitative *in-vitro* test for the detection and identification of IgG or IgA antibodies against SARS-CoV-2 (**s**evere **a**cute **r**espiratory **s**yndrome **co**rona**v**irus-2) in human serum or plasma. *recom*Well SARS-CoV-2 IgG or IgA is a screening test based on the principle of an indirect ELISA. The test can be carried out either manually or automated.

2 Field of application

SARS-CoV-2 belongs to the *Coronaviridae* family and is the causative pathogen for the COVID-19 pandemic. SARS coronaviruses spread primarily via droplets in exhaled air to transmit from person to person. Symptoms range from fever, cough and dyspnoea to pneumonia and acute respiratory distress syndrome and ultimately death in persons with comorbidities. There is currently no medication or vaccine available that can prevent a SARS-CoV-2 associated illness.
IgG or IgA antibodies against SARS-CoV-2 are detected using the *recom*Well SARS-CoV-2 IgG, IgA.

3 Test principle

Highly purified recombinant nucleocapsid protein from SARS-CoV-2 is fixed in the wells of the microtitre plate.

1. Diluted serum or plasma samples are incubated in the wells and specific antibodies to the pathogen antibodies attach to the surface of the wells.
2. Unbound antibodies are then washed away.
3. In a second step, anti-human immunoglobulin antibodies (IgG or IgA), which are coupled to horseradish peroxidase, are incubated in the wells.
4. Unbound conjugate antibodies are then washed away.
5. Specific bound antibody is detected in a colour reaction catalysed by the peroxidase. If an antigen-antibody reaction has taken place, the colour intensity of the solution increases in proportion to the quantity of bound anti-SARS-CoV-2 IgG or IgA antibodies. The intensity of the colour can be measured using a photometer and provides information about the concentration of the anti-SARS-CoV-2 antibodies in the sample.

4 Reagents

4.1 Package contents

The reagents in one pack are sufficient for 96 assays.
Each set of reagents contains:

WASHBUF 10 X	**100 ml** wash buffer (**10× concentrate**), contains phosphate buffer, NaCl and detergent Preservatives: MIT (0.01%) and Oxypyrion (0.1%)
DILUBUF	**125 ml** dilution buffer (**ready to use**), contains protein, detergent and blue dye Preservatives: MIT (0.01%) and Oxypyrion (0.1%)
SUBS TMB	**12 ml** chromogenic substrate tetramethylbenzidine (TMB, **ready to use**)
SOLN STOP	**12 ml** stop solution 24.9% phosphoric acid (H_3PO_4)
INSTRU	**1** instructions for use
EVALFORM	**1** evaluation sheet
TAPE	**2 sheets** sealing film

***recom*Well SARS-CoV-2 IgG** also contains:

MTP	**12×8 well** microtitre plate coated with recombinant SARS-CoV-2 antigen in a vacuum-sealed pouch
CONTROL + IgG	**450 µl** positive control (**purple** cap), contains MIT (0.1%) and Oxypyrion (0.1%)
CONTROL ± IgG	**450 µl** cut-off control (**yellow** cap), contains MIT (0.1%) and Oxypyrion (0.1%)
CONTROL - IgG	**450 µl** negative control (**white** cap), contains MIT (0.1%) and Oxypyrion (0.1%)
CONJ IgG	**500 µl** anti-human IgG conjugate (**101× concentrate**, **red** cap), contains NaN_3 (<0.1%), MIT (<0.01%) and chloracetamide (<0.1%)

***recom*Well SARS-CoV-2 IgA** also contains:

MTP	**12X8 wells** microtitre plate (strip marked **blue**) coated with recombinant SARS-CoV-2 antigen in a vacuum-sealed pouch
CONTROL + IgA	**450 µl** positive control (**brown** cap), contains MIT (0.1%) and Oxypyrion (0.1%)
CONTROL ± IgA	**450 µl** cut-off control (**orange** cap), contains MIT (0.1%) and Oxypyrion (0.1%)
CONTROL - IgA	**450 µl** negative control (**white** cap), contains MIT (0.1%) and Oxypyrion (0.1%)
CONJ IgA	**500 µl** anti-human IgA conjugate (**101× concentrate**, **blue** cap), contains NaN_3 (<0.1%), MIT (<0.01%) and chlorazetamide (<0.1%)

4.2 Additionally required reagents, materials and equipment

- Deionised water (high quality)
- Test tubes
- Vortex mixer or other rotatory device
- 8 channel pipette or washer with pump
- Clean measuring cylinders, 50 ml and 1000 ml
- Micropipettes with single-use tips, 10 µl and 1000 µl
- 10 ml pipette or dispenser
- Incubator 37°C
- Microtitre plate photometer
- Timer
- Disposable protective gloves
- Waste container for biohazardous substances

5 Shelf life and handling

- Store reagents between +2°C and +8°C before and after use; **do not freeze**.
- Before starting the test, allow all reagents to sit at room temperature (+18°C to +25°C) for at least 30 minutes.
- The dilution buffer, wash buffer, substrate and stop solution for the *recom*Well tests can be used for different batches and parameters. In doing so, pay attention to the shelf life of these components.
- The control sera and conjugates are batch specific and must not be used for different parameters or batches.
- Before use, mix the concentrated conjugates, controls and patient samples thoroughly. Avoid foam formation.
- All MIKROGEN microtiter plates are provided with break-apart strips.
- The sealing films are intended for single use.
- The packages have an expiry date, after which a guarantee of quality can no longer be given.
- Keep the kit components away from direct sunlight throughout the test procedure. The substrate solution (TMB) is light sensitive.
- The test must only be performed by trained, authorised and qualified personnel.
- Substantial changes made by the user to the product or the directions for use may compromise the intended purpose of the test specified by Mikrogen.
- Cross-contamination of the patient samples or conjugates in the kit can lead to false test results. Add patient samples and conjugate solution carefully. Make sure that any incubated liquids are not carried over to other wells.
- Automation is possible. Further details are available from Mikrogen.

6 Warnings and safety precautions

- Only use for in-vitro diagnostics.
- All blood products must be treated as potentially infectious.
- The microtitre wells have been coated with inactivated whole-cell lysate, bacterial or viral antigens.
- After adding patient or control material, the microtitre wells must be considered to be potentially infectious and handled accordingly.
- To prepare the control material, blood is used from donors who have been confirmed to have no antibodies to HIV 1/2 or HCV and no HBs antigens. Because an infection cannot be reliably ruled out, however, the control material must be handled with the same care as a patient sample.

- Suitable single-use gloves must be worn throughout the entire test procedure.
- The conjugates and the wash and dilution buffers contain the antimicrobial agents and preservatives sodium azide (NaN_3), MIT (methylisothiazolinone), Oxypyrion and chloroacetamide. Avoid contact with the skin or mucous membranes. Sodium azide can form explosive azides if it comes into contact with heavy metals such as copper and lead.
- Phosphoric acid is an irritant. Use caution and avoid contact with skin and mucous membranes.
- All discarded fluids must be collected. All collection reservoirs must contain suitable disinfectants for inactivation of human pathogens. All reagents and materials coming into contact with potentially infectious samples must be treated with suitable disinfectants or be disposed of according to laboratory guidelines. The concentration specifications and incubation times of the manufacturer must be taken into consideration.
- Use microtitre wells only once.
- Do not replace or mix the reagents with reagents of other manufacturers.
- Read through and carefully follow all instructions before performing the test. Deviations from the test protocol described in the instructions for use can lead to false results.

7 Sampling and preparation

7.1 Sample material

The sample material can be either serum or plasma (EDTA, citrate, heparin, CPD) and must be separated as soon as possible after collection to avoid haemolysis. Microbial contamination of the sample must absolutely be avoided. Non-soluble substances must be removed from the sample prior to incubation. Use of icteric, haemolytic, lipaemic or cloudy samples is not recommended.

Caution!

If the tests are not carried out immediately, the sample material can be stored for up to 2 weeks between +2°C and +8°C. It is possible to store the samples for longer periods at -20°C or lower. Repeated freezing and thawing of the samples is not recommended due to the risk of false results. More than 3 freezing and thawing cycles should be avoided.

7.2 Preparation of solutions

The detection reagents are sufficient for 96 measurements. The quantities specified below apply to the processing of one 8-well microtitre plate strip. If using several microtitre plate strips at the same time, each of the specified quantities must be multiplied by the number of microtitre plate strips used. Device-specific dead volume must be taken into account. Dilution buffer, substrate and stop solution are ready to use.

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

The wash buffer concentrate is diluted **1 + 9** with deionised H_2O. For each 8-well microtitre plate strip, 5 ml concentrate are mixed with 45 ml deionised H_2O. The ready-to-use wash buffer can be stored for four weeks between +2ºC and +8ºC or for one week at room temperature.

7.2.2 *Preparation of the conjugate solution*

For each 8-well microtitre plate strip, 1 ml dilution buffer is added to 10 µl anti-human IgG peroxidase conjugate (red cap) or 10 µl anti-human IgA peroxidase conjugate (blue cap) in a clean tube and then mixed well (dilution **1 + 100**). The conjugate solution must be prepared shortly before use; the ready-to-use conjugate solution must not be stored.

8 Test procedure

No.	Implementation	Note
1	Before starting the test, allow all reagents to sit at +18°C to +25°C (room temperature) for at least 30 minutes.	Before opening, bring the **vacuum-sealed pouch** (containing plate) to room temperature to avoid condensation forming in the wells. Remove the required number of strips, reseal the plate in the pouch and store it in the fridge. Before use, the control sera and patient samples as well as the concentrated conjugates must be mixed thoroughly and then briefly centrifuged if possible to collect any liquid on the base of the tubes.
2	Preparation of the samples and controls Pipette **10 µl** of sample or control into each **1 ml** of the dilution buffer and mix well (dilution **1+100**).	**The samples and controls must always be diluted immediately prior to carrying out the test.** All controls must be run with each assay.
3	Sample incubation Pipette **100 µl** of either diluted sample or diluted control into each well and incubate for **1 hour** at **+37°C**.	At least one value must be prepared for the negative control, positive control and the patient samples. The cut-off control must be prepared in duplicate. There is preferably one cut-off control each at the start and at the end of the series. For manual processing, carefully cover the microtitre plate with fresh sealing film. Use the +37°C incubator.
4 a) b) c)	Wash Carefully remove the sealing film. Remove all fluid from the wells. Fill each well with **300 µl** ready-to-use wash buffer (see 7.2.1) → 8.4b	It is recommended to carry out this step with an appropriate ELISA washer. Be certain all wash buffer is completely removed between wash steps. Aspirate or shake out and tap. Carry out the wash steps 8.4b and 8.4c a total of **four times**.
5	Incubation with conjugate Add **100 µl** diluted conjugate solution (see 7.2.2) and incubate for **30 minutes** at **+37°C**.	For manual processing, carefully cover the microtitre plate with unused sealing film.
6	Wash (see 8.4b and 8.4c)	Carry out the wash steps a total of **four times**.
7	Substrate reaction Pipette **100 µl** ready-to-use substrate solution into each well and incubate for **30 minutes** at **room temperature**. The time is calculated from the time substrate solution is pipetted into the first well.	It is not necessary to cover the plate. Protect from direct sunlight.
8	Stopping the reaction Add **100 µl** ready-to-use stop solution per well.	Do not remove the substrate solution before adding the stop solution! Follow the same pipetting scheme used for pipetting the substrate solution.
9	Absorbance measurement Using a microtitre plate photometer, measure the absorbance of the individual wells at 450 nm and the reference wavelength of 620 nm (620 to 650 nm allowed).	Measure absorbance against an air blank. The reading must be completed within 60 minutes of stopping the reaction.
Caution! **Incubated liquids must not be carried over to other wells. Avoid splashing when removing and attaching the sealing film.**		

9 Results

9.1 Analysis

Cut-off (threshold) = the mean is formed using the absorbance values of the two cut-off controls (at the start and the end of the series).

9.1.1 *Qualitative analysis*

Grey area	Lower limit Upper limit	= cut-off = cut-off + 20% (cut-off ×1.2)
Negative	Samples with absorbance values **below** the grey area	
Borderline	Samples with absorbance values **in** the grey area	
Positive	Samples with absorbance values **above** the grey area	

9.1.2 *Quantitative evaluation*

The corresponding antibody activity in **units per ml** is allocated to the absorbance values using a formula. The measurement unit U/ml is an arbitrary unit that does not have any direct relationships to (international) reference values.

U/ml sample	(absorbance of sample / absorbance of cut-off) × 20
Grey area	Lower 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

Samples with a borderline test result should be tested again. If they are again borderline after the second test, it is recommended to collect and test another sample after some time.

The linearity of the test could be confirmed during the evaluation within the following measurement range:
20 U/ml to 105 U/ml (R^2 = 0.95)

With an absorbance ≥ 3.0 or with a measurement above this linear range, either the result should be given as > 105 U/ml or the sample should be diluted and tested again. We recommend initially using a final dilution of 1:500 and then further dilution steps if necessary.

9.2 Validation – Quality control

The test can be analysed if the following conditions are met:

- The individual absorbance values for the duplicate measurement of the cut-off control do not deviate from their mean by more than 20%.
- Absorbance of the negative control ≤ 0.150
- Absorbance of the cut-off control – absorbance of the negative control ≥ 0.050 ($E_{Cutoff} - E_{neg.\ Contr.} \geq 0.050$)
- Absorbance of the positive control – absorbance of the cut-off control ≥ 0.300 ($E_{pos.\ Contr.} - E_{Cutoff} \geq 0.300$)

The controls are used to validate the test results as defined in the section 'Validation – Quality Control'. Determining specific antibodies relative to the cut-off control in U/ml increases the reproducibility of the results because any fluctuations caused by carrying out the test are integrated. Evaluating the positive control and the negative control is not required to validate the test. However, the evaluation can be carried out for purposes of internal quality control if required. In this case, the results should be within the target range stated on the certificate of analysis or on the label.

9.3 Interpretation schematic

Table 1: Note regarding test interpretation for *recom*Well SARS-CoV-2 IgG, IgA

	Test interpretation
IgG	If IgG antibodies against SARS-CoV-2 are present, this indicates contact with the pathogen. It can be an (acute) / recent or past infection.
IgA	If IgA antibodies against SARS-CoV-2 are present, this may indicate an early phase of the infection. However, acute diagnostics is only possible in connection with a seroconversion or direct detection of the pathogen by RT-PCR.

With the detection of antibodies, a SARS-CoV-2 infection with a positive RT-PCR result can be additionally confirmed and the development of the immune response can be monitored. A seroconversion or a significant increase in IgG titer can therefore indicate an acute infection. Direct detection of the pathogen by RT-PCR is the gold standard.

10 Limits of the method, restrictions

- Serological test results must always be viewed within the context of the clinical presentation. The therapeutic consequences of the serological finding must be related to the clinical data.
- In case of unclear or dubious serological results, repeat testing over the course of the infection is recommended.
- For the diagnosis, in each case the clinical presentation and, if necessary, the medical history must also be considered along with the laboratory values.
- A negative result does not rule out the possibility of a SARS-CoV-2 infection. Particularly in an early stage of infection, antibodies may not be present or may not be present in detectable quantities. If a SARS-CoV-2 infection is clinically suspected and the serological results are negative, RT-PCR (e.g. with *ampli*Cube Coronavirus SARS-CoV-2, art. no. 50143 or 50144 from MIKROGEN) should be carried out and/or after 2 weeks another sample is collected and tested.
- Due to the close relationship between the SARS coronaviruses (SARS-CoV and SARS-CoV-2), a cross-reaction with antibodies against SARS-CoV is possible. Cross-reactions with other human pathogenic coronaviruses (HCoV) cannot be ruled out completely but are less likely.

11 Performance characteristics

11.1 Diagnostic sensitivity

To determine the diagnostic sensitivity for IgG and IgA, 59 or respectively 67 samples from people with RT-PCR confirmed SARS-CoV-2 infection were examined.

Table 2: Diagnostic sensitivity for *recom*Well SARS-CoV-2 IgG

***recom*Well SARS-CoV-2 IgG**	**Days after the onset of symptoms**		
	Early < 12 days	**Middle 12–23 days**	**Late > 23 days**
Positive	6	23	28
Borderline	0	1	0
Negative	1	0	0
Diagnostic sensitivity	**86%**	**100%**	**100%**
	98%		

For samples from day 12 after the start of symptoms, the *recom*Well SARS-CoV-2 IgG has a diagnostic sensitivity of 100%.

Table 3: Diagnostic sensitivity for *recom*Well SARS-CoV-2 IgA

***recom*Well SARS-CoV-2 IgA**	**Days after the onset of symptoms**		
	Early < 12 days	**Middle 12–23 days**	**Late > 23 days**
Positive	2	16	21
Borderline	1	2	7
Negative	3	1	14
Diagnostic sensitivity	**50%**	**95%**	**67%**
	73%		

11.2 Diagnostic specificity

To determine the diagnostic specificity, 300 samples from German blood donors were examined that were collected at different times before the start of the SARS-CoV-2 pandemic.

Table 4: Diagnostic specificity for *recom*Well SARS-CoV-2 IgG, IgA

Blood donors (n = 300)	***recom*Well SARS-CoV-2**	
	IgG	**IgA**
Positive	3	2
Borderline	1	0
Negative	296	298
Diagnostic specificity	**98.7%**	**99.3%**

11.3 Analytical specificity

The analytical specificity is defined as the suitability of the test to precisely determine the analytes in the presence of potential interference factors in the sample matrix or cross-reactions with potentially interfering antibodies.

a) Interference: Control studies on potentially interfering factors have shown that the test performance is not influenced by anticoagulants (sodium citrate, EDTA, heparin, CPD), haemolysis, lipaemia or bilirubinaemia of the sample.

b) Cross-reactions: Potential interference of antibodies against other organisms that can induce clinical symptoms similar to those of a SARS-CoV-2 infection (e.g. seasonal coronaviruses, influenza A/B virus, RSV, Adenoviruses, *Mycoplasma pn.*, *Chlamydia pn.*) were investigated in control studies. In addition, conditions were tested that can be attributed to the development of atypical immune system activity (e.g. EBV, CMV, anti-nuclear autoantibodies, pregnancy, rheumatoid factor). The results of the tests can be seen in Table 5.

Table 5: Testing of cross-reactivity for *recom*Well SARS-CoV-2 IgG, IgA

Sample set (n = 241)	**positive / borderline with *recom*Well SARS-CoV-2**	
	IgG	**IgA**
Seasonal coronaviruses (HCoV) (n = 9)	0/1	0/0
Influenza A virus (n = 9)	1/0	0/0
Influenza B virus (n = 5)	0/0	0/0
Respiratory syncytial virus (RSV) (n = 10)	0/0	0/0
Adenoviruses (n = 6)	1/1	0/0
Mycoplasma pneumoniae (n = 10)	0/0	0/0
Chlamydia pneumoniae (n = 25)	1/0	1/1
Epstein-Barr virus (EBV) (n = 31)	2/0	1/1
Cytomegalovirus (CMV) (n = 11)	2/0	2/0
Autoantibodies positive (n = 15)	0/0	0/0
Pregnant women (n = 60)	0/1	0/0
Rheumatoid factor positive (n = 50)	2/0	1/0

12 Literature

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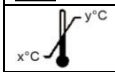
We will be pleased to send you additional literature on request.

13 Explanation of symbols

Σ	Content is sufficient for <n> formulations Number of formulations
WASHBUF 10 X	Wash buffer (10× concentrate)
DILUBUF	Dilution buffer
SUBS TMB	Chromogenic substrate tetramethylbenzidine
SOLN STOP	Stop solution
TAPE	Sealing film
MTP	Microtiter plate
CONTROL + IgG	Positive control IgG
CONTROL ± IgG	Cut-off control IgG
CONTROL - IgG	Negative control IgG
CONJ IgG	Anti-human IgG conjugate
CONTROL + IgA	Positive controls IgA
CONTROL ± IgA	Cut-off controls IgA
CONTROL - IgA	Negative controls IgA
CONJ IgA	Anti-human IgA conjugate
TVALUE	Target value and/or target range in U/ml
EVALFORM	Evaluation sheet
INSTRU	Instructions for use
	Follow the instructions for use
CONT	Contents, contains
IVD	In-vitro diagnostic agent
LOT	Batch/version number
	Do not freeze
REF	Order number
	Use by Expiry date
	Store between x°C and y°C
	Manufacturer

14 Manufacturer and version data

***recom*Well SARS-CoV-2 IgG** ***recom*Well SARS-CoV-2 IgA**	Article no. **7304** Article no. **7305**
Instructions for use Valid from	GARECS003EN 2023-04

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