*recom*Line SARS-CoV-2 IgG

MIKROGEN DIAGNOSTIK

IVD

Instructions for use (English)

1 Purpose

The *recom*Line SARS-CoV-2 IgG is a qualitative test for the detection of IgG antibodies against SARS-CoV-2 (**s**evere **a**cute **r**espiratory **s**yndrome **co**rona**v**irus-2) in human serum or plasma.

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.
The *recom*Line SARS-CoV-2 IgG is a line immunoassay. The separate line-up of individual antigens means that, unlike ELISA, the test allows identification of specific antibodies against the individual antigens of the various coronaviruses. In the test, the following recombinant antigens are used for SARS-CoV-2: nucleocapsid (NP), RBD (receptor-binding domain of the spike protein) and S1 (S1 subunit of the spike protein). Antibodies against seasonal human coronaviruses (HCoV: 229E, NL63, OC43, HKU1) are also detected by the corresponding nucleocapsid antigens (NP).
The *recom*Line SARS-CoV-2 IgG can be used as a confirmatory test to clarify unclear screening results and as a screening test.

3 Test principle

Highly purified recombinant antigens (NP, RBD and S1 from SARS-CoV-2 as well as NP for 229E, NL63, OC43, HKU1) are fixed to nitrocellulose membrane test strips.

1. The test strips are incubated with the diluted serum or plasma sample with specific antibodies binding to the pathogen antigens on the test strips.
2. Unbound antibodies are then washed away.
3. The strips are incubated in a second step with anti-human immunoglobulin antibodies (IgG) that are coupled to horseradish peroxidase.
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, a dark band appears on the strip at the corresponding location.

Control bands are located at the top end of the test strip:

a) The reaction control below the strip number for which every serum/plasma sample must show a reaction.
b) The conjugate controls (IgG, IgA) are used to check the conjugate and strip type used (Ig class specific).
c) 'Cut-off control': The intensity of this band enables an evaluation of the reactivity of the individual antigen bands (see 9.2 Analysis).

4 Reagents

4.1 Package contents

The reagents in one pack are sufficient for 20 assays.
Each set of reagents contains:

WASHBUF A 10 X	**100 ml** wash buffer A (**10× concentrate**) Contains phosphate buffer, NaCl, KCl, detergent, preservative: MIT (0.1%) and Oxypyrion (0.2%)
SUBS TMB	**40 ml** chromogenic substrate tetramethylbenzidine (**TMB, ready-to-use**)
MILKPOW	**5 g** skimmed milk powder
INSTRU	**1** instructions for use
EVALFORM	**1** evaluation sheet
TESTSTR	**2** tubes each with 10 consecutively numbered test strips
CONJ IgG	**500 µl** anti-human IgG conjugate **(100× concentrate, green cap)** From rabbit, contains NaN_3 (<0.1%), MIT (<0.1%) and chloroacetamide (<0.1%)

4.2 Additionally required reagents, materials and equipment

- Incubation trays (can be purchased from MIKROGEN as required)
- Deionised water (high quality)
- Plastic forceps
- Horizontal shaker
- Vortex mixer or other rotatory device
- Vacuum pump or similar device
- Measuring cylinders, 50 ml and 1000 ml
- Micropipettes with single-use tips, 20 µl and 1000 µl
- 10 ml pipette or dispenser
- Timer
- Absorbent paper towels
- Single-use 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 test is carried out at room temperature.
- Wash buffer, milk powder, dilution buffer, conjugates and TMB can be exchanged between the different *recom*Line and/or *recom*Blot test systems if these components carry the same symbol. Note the shelf life of these components when doing so.
- Before use, mix the concentrated reagents and patient sera thoroughly. Avoid foam formation.
- Only open the tubes with the test strips just before use to prevent water condensation. Strips that are not needed must be left in the tube and are continued to be stored at +2°C to +8°C (reseal tube firmly, test strips must not be moist before the start of the test!).
- The strips are identified with a consecutive number and a test abbreviation.
- The packages have an expiry date, after which no further guarantee of quality can 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, test strips and conjugate solution carefully. Make sure that any incubated liquids are not carried over to other wells. Carefully remove liquids.
- The strips must be completely wetted and submersed throughout the entire procedure.
- 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 test strips were manufactured with inactivated whole-cell lysates and/or recombinant bacterial, viral or parasitic antigens.
- After adding patient or control material, the strips must be considered to be potentially infectious and handled appropriately as such.
- Suitable single-use gloves must be worn throughout the entire test procedure.
- The reagents 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 (NaN_3) can form explosive azides if it comes into contact with heavy metals such as copper and lead.
- All aspirated liquids must be collected. All collection reservoirs must contain suitable disinfectants for inactivation of human pathogens or be autoclaved. All reagents and materials that come into contact with potentially infectious samples must be treated with suitable disinfectants or be disposed of according to laboratory guidelines. The concentrations and incubation times specified by the manufacturer must be followed.

- Only use incubation trays once.
- Handle strips carefully with a pair of plastic forceps.
- 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

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

This buffer is required for the serum and conjugate dilution and the wash steps.

Before the dilution, the volume of wash buffer A must be determined for the corresponding number of tests to be carried out.

The skimmed milk powder is first pre-dissolved in wash buffer A concentrate and then this mixture is made up to the final volume with deionised water (dilution 1 + 9). The required quantity for a defined number of test strips is calculated using the following formulae (device-specific dead volumes are not taken into account):

Reagent	Formula	Example: 5 strips
Skimmed milk powder [g]	= strip number × 0.1	0.5 g
Wash buffer A concentrate [ml]	= strip number × 2	10 ml
Deionised water [ml]	= strip number × 18	90 ml
Ready-to-use wash buffer A [ml]	= strip number × 20	100 ml

Ready-to-use wash buffer A can be stored at +2°C to +8°C for four weeks. The ready-to-use wash buffer A has no odour and is slightly turbid.

7.2.2 *Preparation of conjugate solutions*

The conjugate solution must be prepared shortly before use; the ready-to-use conjugate solution must not be stored.

One part of the conjugate concentrate is diluted with 100 parts of the ready-to-use wash buffer A (1 + 100).

The required quantity for a defined number of test strips is calculated using the following formulae:

Reagent	Formula	Example: 5 strips
Conjugate concentration [µl]	= strip number × 20	100 µl
Ready-to-use wash buffer A [ml]	= strip number × 2	10 ml

The conjugate quantities are calculated without dead volumes. Depending on the processing (manually or with a device), please prepare additional conjugate solution for 1 to 3 strips.

8 Test procedure

No.	Implementation	Note
1	Before starting the test, allow all reagents to sit between 18°C and 25°C (room temperature) for at least 30 minutes.	The test is carried out at room temperature.
2	Prepare the test strips Place strips in **2 ml** ready-to-use **wash buffer A** (see 7.2.1).	Do not handle the strips with bare hands – use forceps. The strip number faces upwards. For each strip one well in an incubation tray (see 4.2) is required. The strips must be completed submersed.
3 a) b)	Sample incubation **20 µl** of an undiluted sample (human serum or plasma) are added by pipette to the test strip for each incubation mixture (dilution 1 + 100). Incubate for **1 hour** with gentle shaking.	 Pipette the sample onto one end of the submersed strip in wash buffer A and mix as quickly as possible by gently shaking the incubation bath. Cover the incubation tray with the plastic lid and place on the shaker.
4 a) b) c)	Wash Carefully remove the plastic lid from the incubation tray. Carefully aspirate the serum dilution from the individual wells. Pipette **2 ml** ready-to-use **wash buffer A** (see 7.2.1) into each well, wash for 5 minutes with gentle shaking and then aspirate off the wash buffer A.	Carry out wash steps 8.4a–8.4c a total of three times. Avoid cross-contamination. With automated processing, follow the directions of the device manufacturer for this step.
5	Incubation with conjugate Add **2 ml** ready-to-use **conjugate solution** (see 7.2.2) and incubate for **45 minutes** with gentle shaking.	Cover the incubation tray with the plastic lid and place on the shaker.
6	Wash See section 8.4	Carry out wash steps a total of three times (see 8.4a–8.4c).
7	Substrate reaction Add **1.5 ml substrate solution** and incubate for **8 minutes** with gentle shaking.	
8	Stopping the reaction Remove the substrate solution. Wash at least three times **briefly** with **deionised water**.	
9	Drying the strips Dry the strips before the analysis for **2 hours** between 2 layers of absorbent paper.	Carefully remove the strips from the water with a pair of plastic forceps. Store the strips protected from light.
Caution! **Incubation solutions must not be carried over to other wells. Avoid splashing, particularly when opening and closing the cover.**		

9 Results

Caution:

Do not use the automated interpretation without noting the information described below about the interpretation.

9.1 Validation – Quality control

The test can only be analysed when the following criteria are satisfied:

1. Reaction control band (upper line) is clearly stained, dark band detectable.
2. Antibody class (second band): the IgG conjugate control band must be clearly stained.
3. Cut-off control (third band): weak but visible staining.

9.2 Analysis

The test strips can be analysed visually or with a computer using the *recom*Scan test strip analysis software. The *recom*Scan software is intended to help with test strip interpretation. Additional information and corresponding instructions for computer-aided analysis are available from MIKROGEN upon request. The following instruction refers to the visual analysis.

9.2.1 *Evaluation of the band intensity*

1. Note the date and batch number along with the antibody class that was detected in the attached evaluation form.
2. Enter the sample identification numbers in the evaluation form.
3. Now adhere the associated test strip with a glue stick into the corresponding field in the evaluation form. Align the test strip with the reaction control band at the indicated marker line. Then adhere the test strip to the left of the marking line using transparent adhesive tape (do not stick over the reaction control band!). Adhering the entire test strip with glue stick or adhesive tape can lead to changes in the staining.
4. Now identify the bands for the developed test strips using the printed control strip from the evaluation form and enter these into the evaluation form. Carry out the evaluation of the intensity of the emergent bands separately for the relevant immunoglobulin classes using Table 1.

Table 1: Evaluation of the band intensity relative to the cut-off band

Colour intensity of the bands	Evaluation
No reaction	-
Very low intensity (lower than the cut-off band)	+/-
Low intensity (equivalent to the cut-off band)	+
Strong intensity (stronger than the cut-off band)	++
Very strong intensity	+++

9.3 Interpretation schematic

Table 2: Test interpretation for *recom*Line SARS-CoV-2 IgG

Test interpretation	Antigen reactivity
SARS-CoV-2 IgG positive	One or more SARS-CoV-2-specific antigen bands (NP, RBD and/or S1) are positive, that is, they react with the same (+) or a stronger intensity than the cutoff band (regardless of the reactivity of the HCoV antigens).
SARS-CoV-2 IgG negative	All SARS-CoV-2-specific antigen bands (NP, RBD and S1) are negative, that is, they do not show any bands (−) or bands with a lower (+/−) intensity than the cutoff band (regardless of the reactivity of the HCoV antigens).

The detection of antibodies can, based on current knowledge, provide additional confirmation of a SARS-CoV-2 infection with corresponding positive RT-PCR results and monitor the development of the immune response. Seroconversion or a clear rise in the IgG titre can therefore indicate an acute infection. Direct detection of the pathogen using RT-PCR is considered the gold standard.

Along with SARS-CoV-2, reactivities against the seasonal human coronaviruses (HCoV: 229E, NL63, OC43, HKU1) are also determined. A total seroprevalence of 70%–90% can be expected (see also Table 6). The reactivities of the HCoVs determined do not allow differentiation or any conclusions to be drawn about the immune status regarding the particular coronaviruses or about cross-reactivity with SARS-CoV-2.

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 could not be determined in the evaluation of the *recom*Line SARS-CoV-2 IgG.
- Individual cross-reactions with samples from pregnant women, patients with acute EBV infections, rheumatoid-factor-positive patients and lipaemic samples may occur in rare cases. See also Table 5.
- Dark test strips: Some patient samples can produce a dark generalised or patterned staining over the entire nitrocellulose strip (e.g. with sera from patients with milk protein allergies). There are a number of different factors associated with the patient serum that are responsible for this. Evaluating these strips is generally only possible with limitations. Such ‘inverse’ bands (white bands on a dark background) must be rated negatively. The corresponding serum should be checked using another serological method.

11 Performance characteristics

11.1 Diagnostic sensitivity

To determine the diagnostic sensitivity, 54 samples from people with RT-PCR confirmed SARS-CoV-2 infection were examined.

Table 3: Diagnostic sensitivity for *recom*Line SARS-CoV-2 IgG

***recom*Line SARS-CoV-2 IgG**	**Days after the onset of symptoms**		
	Early < 12 days	**Middle 12–23 days**	**Late > 23 days**
Positive	6	20	26
Negative	1	1	0
Diagnostic sensitivity	**85.7%**	**95.2%**	**100%**
	96.3%		

11.2 Diagnostic specificity

To determine the diagnostic specificity, samples from German blood donors (n = 300) that were collected at different times before the start of the SARS-CoV-2 pandemic as well as potentially cross-reactive (n = 191) or interfering samples (n = 80) were examined.

Table 4: Diagnostic specificity for *recom*Line SARS-CoV-2 IgG

***recom*Line SARS-CoV-2 IgG**	**Blood donors (n = 300)**	**Potentially cross-reactive samples* (n = 191)**	**Potentially interfering samples** (n = 80)**
Positive	1	4	2
Negative	299	187	78
Diagnostic specificity	**99.7%**	**97.9%**	**97.5%**
	98.8%		

* Samples positive for seasonal coronaviruses, influenza A/B virus, RSV, adenoviruses, *Mycoplasma pn., Chlamydia pn.*, EBV, CMV, auto-antibodies as well as from pregnant women.
** Lipaemic, haemolytic and icteric samples, RF-positive samples.

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 interfering 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.*) was 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 evaluation can be seen in Table 5.

Table 5: Testing of cross-reactivities for *recom*Line SARS-CoV-2 IgG

Collective (n = 271)	***recom*Line SARS-CoV-2 IgG**
	Positive
Seasonal coronaviruses (HCoV) (n = 9)	0
Influenza A virus (n = 9)	0
Influenza B virus (n = 5)	0
Respiratory syncytial virus (RSV) (n = 10)	0
Adenoviruses (n = 6)	0
Mycoplasma pneumoniae (n = 10)	0
Chlamydia pneumoniae (n = 25)	0
Epstein-Barr virus (EBV) (n = 31)	2
Cytomegalovirus (CMV) (n = 11)	0
ANA Autoantibodies positive (n = 15)	0
Pregnant women (n = 60)	2
Rheumatoid factor positive (n = 50)	1
Haemolytic samples (n = 10)	0
Lipaemic samples (n = 10)	1
Icteric samples (n = 10)	0

11.4 Prevalence

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

Table 6: Prevalence in Germany before the SARS-CoV-2 pandemic with *recom*Line SARS-CoV-2 IgG

Blood donors (n = 300)	***recom*Line SARS-CoV-2 IgG**
Prevalence	HCoV (229E, NL63, OC43, HKU1)
	81.9%

12 Literature

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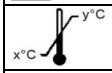
We will be pleased to send you additional literature on request.

13 Explanation of symbols

	Content is sufficient for <n> formulations Number of formulations
WASHBUF A 10 X	Wash buffer A (10× concentrate)
SUBS TMB	Chromogenic substrate tetramethylbenzidine
MILKPOW	Skimmed milk powder
TESTSTR	Test strips
CONJ IgG	Anti-human IgG conjugate
EVALFORM	Evaluation sheet
INSTRU	Instructions for use
	Follow the instructions for use
CONT	Contents, contains
IVD	In-vitro diagnostic agent
LOT	Batch 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*Line SARS-CoV-2 IgG**	Article no. **7374**
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