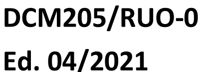
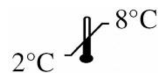
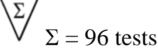


DCM205/RUO-0
Ed. 04/2021

Spike Protein Inhibition Assay (SPIA)

Detection of neutralising antibodies against SARS-CoV-2 in human serum.

RUO LOT See external label   Σ = 96 tests **REF DKO205/RUO**

INTENDED USE
The Spike Protein Inhibition Assay (SPIA) is a competitive immunoenzymatic colorimetric method to detect neutralising antibodies against SARS-CoV-2 in human serum.
Results generated with the SPIA kit are intended for research use only and should not be used for diagnostic purpose.

1. CLINICAL SIGNIFICANCE

The 2019 novel coronavirus, named SARS-CoV-2 is identified as the causative agent of an outbreak of a viral pneumonia or SARS (Severe Acute Respiratory Syndrome) which the World Health Organisation (WHO) subsequently named COVID-19[1]. The viral infection causes a series of respiratory illness including severe respiratory syndrome, indicating the virus most likely infects respiratory epithelial cells and spreads mainly via respiratory tract from human to human[2]. SARS-CoV-2 belongs to the β-coronavirus genus and is similar to the SARS outbreak in 2003 and MERS (Middle East Respiratory Syndrome) in 2012.

The genome of coronavirus encodes four structural proteins including Spike (S), Envelope (E), Membrane (M) and Nucleocapsid (N) proteins. The virus infects and replicates itself within Type II pneumocytes cells and is transmitted via primarily droplet[3].

Receptor-mediated endocytosis is the main process of virus entry to the host cells: S protein contains a receptor binding domain (RBD) involved in the binding of the angiotensin-converting enzyme 2 (ACE2)[4]. ACE2 is a cell-surface receptor that is present in the kidney, blood vessels, heart and importantly, in the lung alveolar type II (AT2) cells which are respiratory tract epithelial cells.
Once the virus gains access inside the target cell, the host immune system recognises the whole virus or its surface epitopes, eliciting an immune response, characterised by elevations in specific antibodies. Those antibodies that are able to bind to the spike protein and prevent it from entering cells by inhibition of the RBD-ACE2 interaction are known as neutralising antibodies (nAb) and the presence of these have been shown to be associated with protection against infection in disease models[5]. While nAb are believed to be important for protection, the level required is not defined. As such a wide range of nAb titres have been reported following SARS-CoV-2 infection and these vary depending on the length of time from infection and the severity of the disease.

2. PRINCIPLE

The principle of the kit is competitive/inhibition ELISA. Anti SARS-CoV-2 neutralising antibodies present in controls and prediluted patient samples compete with a horse-radish peroxidase (HRP) labelled ACE2 receptor for a limited number of SARS-CoV-2 RBD antigens coated on the microwell plate (solid phase). After an incubation phase at 37°C, the bound/free separation is performed by a simple solid-phase washing step. Following the wash step, a substrate solution (TMB) is added and the enzyme HRP in the bound fraction reacts with the Substrate developing a blue colour that becomes yellow upon addition of a stop solution (H_2SO_4). The absorbance of the stopped reaction mixtures are read in a microtiter plate reader, the colour intensity developed is inversely proportional to the inhibition activity of the antibodies in the sample.

3. REAGENTS, MATERIALS AND INSTRUMENTATION

3.1. Reagents and materials supplied in the kit

1. Calibrator (1 vial, 0.5 mL each)
Phosphate buffer 0,1M, NaN_3 < 0,1%
CAL0 **REF DCE002/20506-0**
2. Controls (2 vials, 0.5 mL each)
Phosphate buffer 0,1M, NaN_3 < 0,1%
Control Low **REF DCE045/20501-0**
Control High **REF DCE045/20502-0**
3. Sample Diluent (1 vial, 100 mL)
Phosphate buffer 0,1 M NaN_3 < 0,1%
REF DCE053-0
4. Conjugate (1 vial, 7.5 mL)
hACE2 conjugated with horseradish peroxidase (HRP), BSA 0,1%, Proclin < 0,0015% **REF DCE002/20502-0**
5. Coated Microplate (1 breakable microplate)
Microplate coated with SARS-CoV-2 Spike RBD antigen
REF DCE002/20503-0
6. TMB Substrate (1 vial, 15 mL)
H_2O_2-TMB 0.26 g/L *(avoid any skin contact)*
REF DCE004-0
7. Stop Solution (1 vial, 15 mL)
Sulphuric acid 0.15M *(avoid any skin contact)*
REF DCE005-0
8. 10X Conc. Wash Solution (1 vial, 50 mL)
Phosphate buffer 0,2M, pH 7.4 **REF DCE054-0**

3.2. Reagents necessary not supplied

Distilled water.

3.3. Auxiliary materials and instrumentation

Automatic dispenser.
Microplate reader (450 nm, 620-630 nm).

Notes
Store all reagents between 2÷8°C in the dark.
Open the bag of reagent 5 (Coated Microplate) only when it is at room temperature and close it immediately after use; once opened, it is stable until expiry date of the kit.

4. WARNINGS

- This kit is intended for research use only by professional persons. Not for internal or external use in Humans or Animals.
- Use appropriate personal protective equipment while working with the reagents provided.
- Follow Good Laboratory Practice (GLP) for handling blood products.
- Material of animal origin used in the preparation of the kit has been obtained from animals certified as healthy and the bovine protein has been obtained from countries not infected by BSE, but these materials should be handled as potentially infectious.
- Some reagents contain small amounts of Sodium Azide (NaN_3) or Proclin 300® as preservatives. Avoid the contact with skin or mucosa.
- Sodium Azide may be toxic if ingested or absorbed through the skin or eyes; moreover it may react with lead or copper plumbing to form potentially explosive metal azides. If you use a sink to remove the reagents, allow scroll through large amounts of water to prevent azide build-up.
- The TMB Substrate contains an irritant, which may be harmful if inhaled, ingested or absorbed through the skin. To prevent injury, avoid inhalation, ingestion or contact with skin and eyes.
- The Stop Solution consists of a diluted sulphuric acid solution. Sulphuric acid is poisonous and corrosive and can be toxic if ingested. To prevent chemical burns, avoid contact with skin and eyes.
- Avoid the exposure of reagent TMB/H_2O_2 to directed sunlight, metals or oxidants. Do not freeze the solution.

5. PRECAUTIONS

- Please adhere strictly to the sequence of pipetting steps provided in this protocol. The performance data represented here were obtained using specific reagents listed in this Instruction For Use.
- All reagents should be stored refrigerated at 2-8°C in their original container. The reagents are stable until the expiry date when stored and handled as indicated.
- Allow all kit components and specimens to reach room temperature (22-28°C) and mix well prior to use.
- Do not interchange kit components from different lots. The expiry date printed on box and vials labels must be observed. Do not use any kit component beyond their expiry date.
- If you use automated equipment, the user has the responsibility to make sure that the kit has been appropriately tested.
- The incomplete or inaccurate liquid removal from the wells could influence the assay precision and/or increase the background. To improve the performance of the kit on automatic systems is recommended to increase the number of washes.
- It is important that the time of reaction in each well is held constant for reproducible results. Pipetting of samples should not extend beyond ten minutes to avoid assay drift. If more than 10 minutes are needed, follow the same order of dispensation. If more than one plate is used, it is recommended to repeat the dose response curve in each plate
- Addition of the TMB Substrate solution initiates a kinetic reaction, which is terminated by the addition of the Stop Solution. Therefore, the TMB Substrate and the Stop Solution should be added in the same sequence to eliminate any time deviation during the reaction.
- Observe the guidelines for performing quality control in laboratories by assaying controls and/or pooled sera.
- Maximum precision is required for reconstitution and dispensation of the reagents.
- Samples microbiologically contaminated, highly lipemeic or haemolysed should not be used in the assay.
- Plate readers measure vertically. Do not touch the bottom of the wells.

6. PROCEDURE

6.1. Preparation of the Calibrator and Controls

Calibrator and controls are ready to use. Once opened, Calibrator and controls are stable for 6 months at 2-8°C.

6.2. Preparation of the Sample

The determination of SARS-CoV-2 neutralising antibodies should be performed on human serum samples.

It is recommended that all serum samples are prediluted 1:5 with sample diluent; for example 25 µL of sample should be diluted with 100 µL of sample diluent. Higher dilution may be required for patients with elevated antibody titre.

6.3. Preparation of the Wash Solution

Dilute the content of each vial of the "10X Conc. Wash Solution" with distilled water to a final volume of 500 mL prior to use. For smaller volumes respect the 1:10 dilution ratio. The diluted wash solution is stable for 30 days at 2-8°C.
In concentrated wash solution is possible to observe the presence of crystals; in this case mix at room temperature until the complete dissolution of crystals; for greater accuracy, dilute the whole bottle of concentrated wash solution to 500 mL, taking care to transfer completely the crystals, then mix until crystals are completely dissolved.

6.4. Procedure

- **Allow all reagents to reach room temperature (22-28°C) for at least 30 minutes.** At the end of the assay immediately store the reagents at 2-8°C: avoid long exposure to room temperature.
- Unused coated microwell strips should be released securely in the foil pouch containing desiccant and stored at 2-8°C.
- To avoid potential microbial and/or chemical contamination, unused reagents should never be transferred into the original vials.
- As it is necessary to perform the determination in duplicate in order to improve accuracy of the test results,

prepare two wells for calibrator (C_0), two for each Control, two for each sample, one for Blank.

<table>
<tr><th>Reagent</th><th>Calibrator / Controls</th><th>Diluted Sample</th><th>Blank</th></tr>
<tr><td>Calibrator / Controls</td><td>50 µL</td><td></td><td></td></tr>
<tr><td>Diluted Sample</td><td></td><td>50 µL</td><td></td></tr>
<tr><td>Conjugate</td><td>50 µL</td><td>50 µL</td><td></td></tr>
<tr><td colspan="4">Incubate at 37°C for 90 minutes.
Remove the content from each well, wash the wells 3 times with 300 µL of diluted wash solution.
Important note: during each washing step, gently shake the plate for 5 seconds and remove excess solution by tapping the inverted plate on an absorbent paper towel.
Automatic washer: if you use automated equipment, wash the wells at least 5 times.</td></tr>
<tr><td>TMB Substrate</td><td>100 µL</td><td>100 µL</td><td>100 µL</td></tr>
<tr><td colspan="4">Incubate at room temperature (22-28°C) for 15 minutes in the dark.</td></tr>
<tr><td>Stop Solution</td><td>100 µL</td><td>100 µL</td><td>100 µL</td></tr>
<tr><td colspan="4">Shake the microplate gently
Read the absorbance (E) at 450 nm against a reference wavelength of 620-630 nm or against Blank within 5 minutes.</td></tr>
</table>

7. QUALITY CONTROL

Each laboratory should assay controls at appropriate levels for monitoring assay performance. These controls should be treated as unknowns and results determined in every test procedure performed.

Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8. CALCULATION OF RESULTS

Calculate the mean of the absorbances (Em) from the duplicates for each test sample. Inhibition value is calculated as detailed below:

$$\% \text{ Inhibition} = [1 - (B/B_0)] \times 100$$

B= absorbance reading of the sample* or control
B_0= absorbance reading of the CAL0

*If sample absorbance value is higher than CAL0 absorbance value with resulting calculated %inhibition as negative value, the result is to be interpreted as 0%

9. RESULTS FROM INTERNAL EVALUATION

A comparison test against a commercial kit for the detection of anti SARS-CoV-2 S1/S2 IgG was performed on 150 sera (50 sera from donors with confirmed COVID-19 diagnosis and 100 pre-pandemic sera). The cut-off for SPIA results interpretation was set as following:

% inhibition	Result
≥ 30%	Positive
<30%	Negative

From this comparison, sensitivity and specificity of the SPIA assay resulted respectively 96% and 99%.

This study was performed for research purpose only. Users should set up their own cut-off for result interpretation based on their own patient serum panels.
Results of the SPIA assay must never be used for diagnostic purpose.

10. WASTE MANAGEMENT

Reagents must be disposed off in accordance with local regulations.

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2. Hu, B., Guo, H., Zhou, P. *et al.* Characteristics of SARS-CoV-2 and COVID-19. *Nat Rev Microbiol* 19, 141–154 (2021).Hussain A, Kaler J, Tabrez E, Tabrez S, Tabrez SSM. Novel COVID-19: A Comprehensive Review of Transmission, Manifestation, and Pathogenesis. *Cureus*. 2020;12(5):e8184.
3. Tai W, He L, Zhang X, Pu J, Voronin D, Jiang S, Zhou Y, Du L. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. Cell Mol Immunol. 2020 Jun;17(6):613-620.
4. Jiang S, Zhang X, Yang Y, Hotez PJ, Du L. Neutralizing antibodies for the treatment of COVID-19. Nat Biomed Eng. 2020 Dec;4(12):1134-1139.
5. Rogers TF, Zhao F, Huang D, *et al.* Isolation of potent SARS-CoV-2 neutralizing antibodies and protection from disease in a small animal model. Science. 2020 Aug 21;369(6506):956-963.

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DiaMetra	Packaging Information Sheet	Mod. PIS000-1

Symbol	Lang.	Meaning	Symbol	Lang.	Meaning
REF	DE ES FR GB IT PT	Bestellnummer Nûmero de catálogo Référéncès du catalogue Catalogue number Numero di Catalogo Número do catálogo		DE ES FR GB IT PT	Hergestellt von Elaborado por Fabriqué par Manufacturer Produttore Produzido por
	DE ES FR GB IT PT	Achtung, Begleitdokumente Precaución, consulte los documentos adjuntos Attention, veuillez consulter les documents d'accompagnement Caution, consult accompanying documents Attenzione, consultare la documentazione allegata Atenção,consultar os documentos de acompanhamento	yyyy-mm	DE ES FR GB IT PT	Herstellungs datum Fecha de fabricacion Date de fabrication Date of manufacture Data di produzione Data de produção
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	DE ES FR GB IT PT	Gebrauchsanweisung beachten Consultar las instrucciones Consulter le mode d'emploi Consult instructions for use Consultare le istruzioni per l'uso Consultar instruções para uso	LOT	DE ES FR GB IT PT	Chargenbezeichnung Codigo de lote Numero de lot Batch code Codice del lotto Codigo do lote
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SUGGERIMENTI PER LA RISOLUZIONE DEI PROBLEMI/TROUBLESHOOTING

ERRORE CAUSE POSSIBILI/ SUGGERIMENTI
Nessuna reazione colorimetrica del saggio
- mancata dispensazione del coniugato
- contaminazione del coniugato e/o del Substrato
- errori nell'esecuzione del saggio (es. Dispensazione accidentale dei reagenti in sequenza errata o provenienti da flaconi sbagliati, etc.)

Reazione troppo blanda (OD troppo basse)
- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo breve, temperatura di incubazione troppa bassa

Reazione troppo intensa (OD troppo alte)
- coniugato non idoneo (es. non proveniente dal kit originale)
- tempo di incubazione troppo lungo, temperatura di incubazione troppa alta
- qualità scadente dell'acqua usata per la soluzione di lavaggio (basso grado di deionizzazione,)
- lavaggi insufficienti (coniugato non completamente rimosso)

Valori inspiegabilmente fuori scala
- contaminazione di pipette, puntali o contenitori- lavaggi insufficienti (coniugato non completamente rimosso)

CV% intrasaggio elevato
- reagenti e/o strip non portate a temperatura ambiente prima dell'uso
- il lavatore per micropiastre non lava correttamente (suggerimento: pulire la testa del lavatore)

CV% intersaggio elevato
- condizioni di incubazione non costanti (tempo o temperatura)
- controlli e campioni non dispensati allo stesso tempo (con gli stessi intervalli) (controllare la sequenza di dispensazione)
- variabilità intrinseca degli operatori

ERROR POSSIBLE CAUSES / SUGGESTIONS
No colorimetric reaction
- no conjugate pipetted reaction after addition
- contamination of conjugates and/or of substrate
- errors in performing the assay procedure (e.g. accidental pipetting of reagents in a wrong sequence or from the wrong vial, etc.)

Too low reaction (too low ODs)
- incorrect conjugate (e.g. not from original kit)
- incubation time too short, incubation temperature too low

Too high reaction (too high ODs)
- incorrect conjugate (e.g. not from original kit)
- incubation time too long, incubation temperature too high
- water quality for wash buffer insufficient (low grade of deionization)
- insufficient washing (conjugates not properly removed)

Unexplainable outliers
- contamination of pipettes, tips or containers

insufficient washing (conjugates not properly removed) too high within-run
- reagents and/or strips not pre-warmed to CV% Room Temperature prior to use
- plate washer is not washing correctly (suggestion: clean washer head)

too high between-run - incubation conditions not constant (time, CV % temperature)
- controls and samples not dispensed at the same time (with the same intervals) (check pipetting order)
- person-related variation

ERROR / POSIBLES CAUSAS / SUGERENCIAS

No se produce ninguna reacción colorimétrica del ensayo
- no se ha dispensado el conjugado
- contaminación del conjugado y/o del substrato
- errores en la ejecución del ensayo (p. ej., dispensación accidental de los reactivos en orden incorrecto o procedentes de frascos equivocados, etc.)

Reacción escasa (DO demasiado bajas)
- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado corto, temperatura de incubación demasiado baja

Reacción demasiado intensa (DO demasiado altas)
- conjugado no idóneo (p. ej., no procedente del kit original)
- tiempo de incubación demasiado largo, temperatura de incubación demasiado alta
- calidad escasa del agua usada para la solución de lavado (bajo grado de desionización)
- lavados insuficientes (el conjugado no se ha retirado completamente)

Valores inexplicablemente fuera de escala
- contaminación de pipetas, puntas o contenedores- lavados insuficientes (el conjugado no se ha retirado completamente)

CV% intraensayo elevado
- los reactivos y/o tiras no se encontraban a temperatura ambiente antes del uso
- el lavador de microplacas no funciona correctamente (sugerencia: limpiar el cabezal del lavador)

CV% interensayo elevado
- condiciones de incubación no constantes (tiempo o temperatura)
- controles y muestras no dispensados al mismo tiempo (con los mismos intervalos) (controlar la secuencia de dispensación)
- variación en función de los operadores

ERREUR CAUSES POSSIBLES / SUGGESTIONS

Aucune réaction colorimétrique de l'essai
- non distribution du conjugué
- contamination du conjugué et/ou du substrat
- erreurs dans l'exécution du dosage (par ex., distribution accidentelle des réactifs dans le mauvais ordre ou en provenance des mauvais flacons, etc.)

Réaction trop faible (DO trop basse)
- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop court, température d'incubation trop basse

Réaction trop intense (DO trop élevée)
- conjugué non approprié (par ex., ne provenant pas du coffret original)
- temps d'incubation trop long, température d'incubation trop élevée
- mauvaise qualité de l'eau utilisée pour la solution de lavage (bas degré de déionisation)
- lavages insuffisants (conjugué non entièrement éliminé)

Valeurs inexplicablement hors plage
- contamination des pipettes, embouts ou récipients - lavages insuffisants (conjugué non entièrement éliminé)

CV% intra-essai élevé
- les réactifs et/ou les bandes n'ont pas atteint la température ambiante avant usage
- le laveur de microplaques ne lave pas correctement (suggestion : nettoyer la tête du laveur)

CV% inter-essai élevé
- conditions d'incubation non constantes (temps ou température)
- contrôles et échantillons non distribués en même temps (avec les mêmes intervalles) (contrôler l'ordre de distribution)
- variabilité intrinsèque des opérateurs