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TECHNOZYM anti SARS-CoV-2 NP IgG ELISA 96T.

ENG
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RUO

TECHNOZYM anti SARS-CoV-2 NP IgG ELISA - English

INTENDED USE

The TECHNOZYM anti SARS-CoV-2 NP IgG ELISA is a quantitative test for detection of IgG antibodies to SARS-CoV-2 for use in research activities, using venous-drawn fresh and/or frozen human serum or plasma. The chromogenic assay is performed on microplate readers or using automated ELISA processing instruments capable of reading a wavelength of 450 nm.

The TECHNOZYM anti SARS-CoV-2 NP IgG ELISA is intended for use in laboratories by professionals, qualified to perform ELISA-based assays.

SUMMARY

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is the virus that causes corona virus disease 2019 (COVID-19). The SARS-CoV-2 is a single stranded RNA coronavirus and causes respiratory infections. Coronaviruses are composed of spike protein, hemagglutinin-esterase dimer, a membrane glycoprotein, an envelope protein a nucleocapsid and RNA. Results suggest that the spike protein retains sufficient affinity to the Angiotensin converting enzyme 2 (ACE2) receptor to use it as a mechanism of cell entry. Human transmission of coronaviruses is primarily thought to occur among close contacts via respiratory droplets generated by sneezing or coughing. IgG is the most abundantly found immunoglobulin to be produced in response to an antigen and will be maintained in the body after initial exposure for long term response. Serological assays can be useful in identifying if people have been exposed to the virus.

The TECHNOZYM anti SARS-CoV-2 NP IgG ELISA uses a recombinant nucleocapsid protein (NP) protein of the SARS-CoV-2 virus to detect antibodies against SARS-CoV-2 in samples and to quantify an immunological response.

REAGENTS

The TECHNOZYM SARS-CoV-2 NP IgG ELISA contains:

	Reagent / Content	Description
12 x 8 wells	ELISA test strips	Microtiter plate coated with recombinant SARS-CoV-2 NP protein; the drying agent is supplied in an aluminum bag.
5 x 0.5 mL	Calibrator serum	Numbered from 1 to 5; lyophilized, with lot-specific concentrations (values see batch table)
2 x 0.5 mL	Control serum	Positive and negative control serum, lyophilized, with lot- specific concentrations (values see batch table)
1 x 90 mL	Incubation buffer (=sample dilution buffer)	Contains stabilizer protein; 0.05 % proclin and dye; liquid; ready to use
1 x 0.3 mL	Conjugate	Anti-human IgG POX, dyed blue; liquid
1 x 12 mL	Chromogenic substrate TMB	Tetramethylbenzidine substrate; liquid, ready to use
1 x 80 mL	Washing buffer concentrate	Contains detergent; 0.01 % merthiolate, liquid
1 x 12 mL	Stop solution	Sulphuric acid 0.5 M, liquid, ready to use
2 pcs	Adhesive film	For ELISA test strips

Material required (not supplied with the kit)

- Distilled water
- Test tubes for diluting samples
- Measuring cylinder (1000 mL)
- Precision pipettes (10,100 and 1000 µL)
- Variable pipette (1000 µL)
- Multichannel and/or dispensing pipettes (100 and 200 µL)
- ELISA washer or multichannel pipette
- ELISA reader with 450 nm filter, with a 620 nm reference filter if available
- Laboratory timer

Warning and precautions

- RUO for research use only.
- This kit is intended for use by personnel trained in laboratory procedures and universal precautions, for the use of chemicals and potentially biohazardous substances must be applied.
- All human blood or serum products, as well as test samples must be considered as potentially infectious. They have to be handled with appropriate care and in strict observance of safety regulations. The rules pertaining to disposal are the same as applied to disposing hospital waste.
- Calibrators and control sera are made from human blood and any individual serum involved in the procedure is HbsAg, HIV 1/2 Ab and HCV-Ab-negative as tested by FDA approved methods. However, all human blood products should be handled as potentially infectious material.
- Get a Material Safety Data Sheet for this product from www.technoclone.com.

Symbol	Warning and Precautions	Product
	H315 causes skin irritation P264 wash hands thoroughly after handling Contains sulphuric acid	Stop solution
	H317 may cause an allergic skin reaction P280 wear protective gloves Contains Methylisothiazol.	Incubation buffer

Stability and storage

The expiry date printed on the labels is only applicable to storage of the unopened containers at 2…8 °C.

Stability opened / in use:

Material / Reagent	State	Storage	Stability
ELISA test strips	After opening	2…8 °C with adhesive film in aluminum bag with drying agent	Expiry date
Calibrators, control sera	After reconstitution	≤ -20 °C	6 months
Incubation buffer / sample dilution buffer	After opening	2...8 °C	2 months
Conjugate	After opening	2...8 °C	6 months
	Working solution	Room temperature (18…25 °C)	60 minutes
Chromogenic substrate TMB	After opening	2…8 °C	Expiry date
Wash Buffer (10-fold concentrate)	After opening	2...8 °C	6 months
Washing Buffer	1+11.5 dilution of concentrate	2...8 °C	3 weeks
Stop Solution	After opening	2...8 °C	Expiry date

TEST PROCEDURE

Preparation of serum samples

Sample material: Human serum, Lithium heparin plasma or EDTA plasma has been tested.

Serum or plasma samples are collected and treated using standard tubes according to the tube manufacturer´s recommendations.

Although samples should only be used on the same day, serum samples may be stored up to three days at room temperature, or seven days at 2-8 °C. At -20 °C they can be stored for several months. Samples may not be frozen and thawed several times.

Thaw frozen samples rapidly at 37 °C and centrifuge if necessary. Gently mix before testing. After thawing, the assay must be performed within 2 hours. Samples may be frozen once at -70 °C.

When freezing samples the minimum volume should be 150 µL!

- *Sample dilution:* Dilute sample 1:400 in incubation buffer.
  Example: Step 1: 20 µL sample + 380 µL incubation (=sample dilution) buffer.
  20 µL of mixture diluted in step 1 + 380 µL incubation (=sample dilution) buffer.

Preparation of reagents

Before starting the test, all the required components are to be brought to room temperature.

When reconstituting material, mixing reagents or buffers avoid foaming.

- *Washing buffer*: Dilute 1 part by volume washing buffer concentrate with 11.5 parts by volume distilled water (1+11.5). Mix well! (diluted washing buffer concentrate = washing buffer). There may be crystalline precipitations which will dissolve at 37 °C within 10 minutes.
- *Calibrators and control material:* Calibrators and controls are reconstituted with 500 µL distilled water and mixed for 10 seconds, after a reconstitution time of 15 minutes. Reconstituted components are clear.
  Calibrators and controls are used undiluted.
  When freezing calibrators or control material the minimum volume should be 150 µL!
- *Conjugate working solution:* Dilute 1 part by volume conjugate with 50 parts by volume incubation buffer (1+50).

Performance of the test

SAMPLE INCUBATION (reference 1,2,8)	Pipette calibrators, control plasmas, diluted samples into test wells	100 µL
	Incubate at room temperature	60 minutes
WASHING (reference 1,3,4)	Washing buffer	3 x 200 µL
CONJUGATE REACTION (reference 1,2,8)	Pipette conjugate working solution into wells	100 µL
	Incubate at room temperature	60 minutes
WASHING (reference 1,3,4)	Washing buffer	3 x 200 µL
SUBSTRATE REACTION (reference 1,2,8)	Pipette Substrate solution into test wells	100 µL
	Incubate at room temperature	15 minutes
STOPPING (reference 1,2)	Pipette stopping solution into wells	100 µL
MEASURING (reference 7)	ELISA-Reader, 450 nm	shake 10 sec., measure within 10 min.

References

1. Reagents of different lots must not be combined.
2. Precision and performance, among others, essentially depend on the following factors:
   - Thorough mixing of all substances used for dilution, 10 sec. with vortex mixer.
   - Run calibrators, controls and samples in duplicates.
   - Incubate at indicated temperature (room temperature 18...25 °C).
   - Strict observance of the order of pipetting and of the time element as indicated.
   - The time for sample incubation, conjugate and substrate reaction as indicated, starts after pipetting the last sample. Incubation times should not vary by more than ± 5 %.
   - During sample incubation and conjugate reaction, the time for pipetting calibrators / control plasmas / samples and / or conjugate solutions must not exceed 60 seconds per ELISA test strip (8 wells).
   - During substrate reactions and at stopping, the time needed for pipetting the substrate and / or the stopping solution must not exceed 10 seconds per ELISA test strip. Short pipetting times may be secured by using multichannel pipettes.
   - Use incubation buffer from actual kit box, do not use incubation buffer left from previous boxes. Keep incubation buffer free from contaminants.
3. Label / number strips with a water-resistant pen, in case the strips accidentally fall out of the frame during testing.
4. After the last washing, wells must be aspirated thoroughly, turned upside down and positioned on a blotting paper. The last remnants must be removed by gentle tapping.
5. A calibration curve has to be created for every assay.
6. No agitation is required during each reaction step.
7. By measuring the difference in wavelength at 450 nm and 620 nm, the precision of the test is increased.
8. For every incubation step, the test plate has to be covered with plate sealer.

LIMITATION OF THE TEST

Anti SARS-CoV-2 NP IgG results are not affected by hemoglobin up to 500 mg/dL, bilirubin up to 40 mg/dL, lipemia up to 1400 mg/dL Intralipid™ and rheumatoid factor up to 620 IU/mL.

INTERPRETATION OF RESULTS

Anti SARS-CoV-2 NP IgG results are reported in U/mL.

CALCULATION OF RESULTS

Setting up a reference curve:

X axis: anti SARS-CoV-2 NP IgG [U/mL]

Y axis: Extinction at 450 nm

Graph plot is linear-linear with a best fit.

Assessment of reference curve:

The validity of the test may be checked on the basis of the calculated control values.

Measuring concentration of samples:

Read off the concentration from the reference curve.

If there are samples, with extinction coefficients higher than the extinction of the highest point on the calibration curve, they have to be pre diluted with reaction buffer (1+1 or 1+3). The measured concentration then has to be multiplied with the dilution factor 2 or 4, respectively.

REFERENCE RANGE

Normal range for SARS-CoV-2 NP IgG: 0.00 – 5.00 U/mL.

It is recommended that individual laboratories establish their own normal range.

For the use of this test in prevalence studies, a higher cut-off value (8.00 U/mL) should be employed to further increase specificity. Additionaly, positive results can then be confirmed by TECHNOZYM SARS-CoV-2 RBD ELISA, to rule out false positive results.

PERFORMANCE CHARACTERISTICS

Performance data are given below. Results obtained in individual laboratories may differ.

Performance

A single center method evaluation was performed with samples covering the whole assay range using TECHNOZYM anti SARS-CoV-2-NP IgG comparing to other commercially available anti SARS-CoV-2 testkits based on a cut-off of 5.00 U/mL.

Days Post Symptom Onset	n	Positive	Negative	Positive Agreement	Median value of positive
<5	34	5	29	14.7%	21.3
5-10	35	16	19	45.7%	32.0
11-15	17	13	4	76.5%	78.6
>15	18	18	0	100%	202.6

Negative Agreement

	N	Positive	Negative	Negative Agreement
Pre-COVID19	200	0	200	100%
ICU	256	1	255	99.6%
Total	456	1	455	99.8%

Limit of detection and assay range

The upper limit of the assay range may vary with each lot of kit depending on the assayed value of the calibrator plasma supplied in the kit. Samples with values outside the range of the reference curve should be re-tested at an appropriate dilution to obtain accurate results.

STANDARDISATION

The calibrators and controls are traceable to a specific antibody against the SARS-CoV-2 receptor binding domain (CR3022). One unit is equivalent to the response of 100 ng/mL CR3022 on a RBD coated plate.

LITERATURE

Please contact Technoclone www.technoclone.com or your local distributor.

EDITORIAL NOTE

This document is available in several languages. The translations have been done using the master document in English. In the event of doubts or discrepancies, the wording in the master document in English shall take precedence.

 Manufacturer

LOT lot

 Storage temperature

 Determinations

 Expiry date

REF Catalogue

 Consult instructions for use

GTIN Global Trade Item Number

 Biological risk

BUF Reaction buffer

RUO Research use only

CAL Calibrator

INC Incubation buffer

CONT Control

STAB stable peroxide solution

CONJ Conjugate /

SUB substrate

DIL Dilute or disolve in

STOP stop solution

MTP Microtiter plate

RTU Ready to use

WASH Washing solution concentrate

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