



COVID-19 Human IgM IgG Assay Kit

Catalog Number KA5826

96 assays

Version: 01

Intended for research use only

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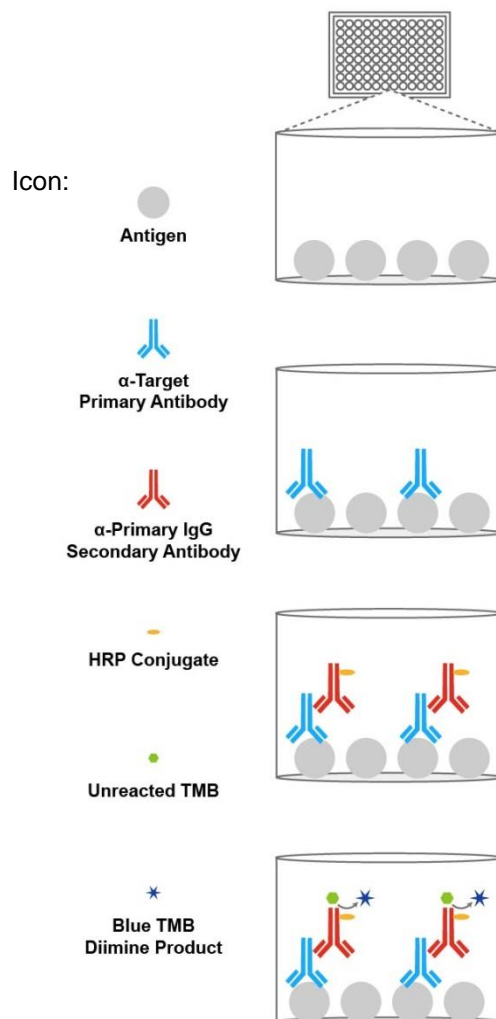
Introduction

Intended Use

COVID-19 Human IgM IgG Assay Kit is an indirect enzyme-linked immunoassay (ELISA) for detection of human IgM and IgG antibodies against COVID-19 virus in human serum.

Principle of the Assay

Qualitative determination of target protein concentration is achieved by an indirect ELISA format. In essence, COVID-19 spike protein is added to the microtiter wells. The COVID-19 spike protein is captured by target-specific primary (1°) antibodies while the HRP-conjugated secondary (2°) antibodies bind the Fc region of the 1° antibody. Through this binding, the HRP enzyme conjugated to the 2° antibody can catalyze a colorimetric reaction upon substrate addition.



General Information

Materials Supplied

List of component

Component	Amount
96-Well COVID-19 Spike Protein Coated Microplate	2 Plates
Anti-human IgM antibody (HRP)	10 μ L
Anti-human IgG antibody (HRP)	10 μ L
10x PBST	100 mL
TMB Reagent	20 mL
Stop Solution	20 mL

Storage Instruction

Upon receipt, the 96-Well COVID-19 Spike Protein Coated Microplate and TMB Reagent should be stored at 4°C. 10x PBST and Stop Solution should be stored at room temperature. The Anti-human IgM antibody (HRP) and Anti-human IgG antibody (HRP) should be stored at -20°C. Avoid cycles of freezing and thawing.

Materials Required but Not Supplied

The following materials and equipment are NOT provided in this kit but are necessary to successfully conduct the experiment:

- ✓ Microplate reader able to measure absorbance at 450 nm.
- ✓ Micropipettes with capability of measuring volumes ranging from 1 μ L to 1 mL.
- ✓ Deionized or sterile water.
- ✓ Squirt bottle, manifold dispenser, multichannel pipette reservoir or automated microplate washer.
- ✓ Graph paper or computer software capable of generating or displaying logarithmic functions.
- ✓ Absorbent papers or vacuum aspirator.
- ✓ Test tubes or microfuge tubes capable of storing ≥ 1 mL.
- ✓ ELISA shaker.
- ✓ 1x PBST
10x PBST dilute to 1x before use.
- ✓ Blocking Solution
5% Skim milk in PBST (ex. 5g skim milk solves in 100ml 1x PBST)
- ✓ Standard/Sample/ Detecting antibody Diluent
2% Skim milk in PBST (ex. 2g skim milk solves in 100ml 1x PBST)

Precautions for Use

- Assay Restrictions
 - ✓ This ELISA kit is intended for research purposes only, NOT diagnostic or clinical procedures of any kind.
 - ✓ Materials included in this kit should NOT be used past the expiration date on the kit label.
 - ✓ Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or substrates from any other kits.
 - ✓ Variations in pipetting technique, washing technique, operator laboratory technique, kit age, incubation time or temperature may cause differences in binding affinity of the materials provided.

- Health and safety precautions
 - ✓ Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin.
 - ✓ Stop Solution contains 1N HCl and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate or strips.

Assay Procedure

Reagent Preparation

Note: Please remember to allow all solutions to warm up to room temperature prior to use.

- Human Serum Diluent
Perform a 1:2000 dilution in Standard/Sample/ Detecting antibody Diluent
- Anti-human IgG antibody (HRP) Diluent
Perform a 1:30000 dilution in Standard/Sample/ Detecting antibody Diluent
- Anti-human IgM antibody (HRP) Diluent
Perform a 1:30000 dilution in Standard/Sample/ Detecting antibody Diluent

Assay Procedure

Note: Please read the whole manual before performing the experiment.

For Human IgG Assay

1. Wash the 96-Well COVID-19 Spike Protein Coated Microplate with 1x PBST for 30 second at a time, with gentle shaking on the shaker.
2. Add 200 μ L of Blocking Solution and incubate for 1 hour at room temperature.
3. Wash 2 times with 200 μ L of 1x PBST for 30 second at a time on the shaker.
4. Add 100 μ L of Human Serum Diluent to the corresponding wells, cover with parafilm and incubate for 1 hour at room temperature.
5. Wash 5 times with 200 μ L of 1x PBST for 30 second at a time on the shaker.
6. Add 80 μ L of Anti-human **IgG** antibody (HRP) Diluent to corresponding wells and incubate for 1 hour at room temperature with gentle shaking on the shaker.
7. Wash 5 times with 200 μ L of 1x PBST for 30 second at a time on the shaker.
8. Add 100 μ L of TMB Reagent to each well and incubate for 6 minutes at room temperature in the dark with gentle shaking on the shaker. TMB Reagent should be return to room temperature before use *Note: TMB Reagent is a light-sensitive reagent. Keep away from light.*
9. Add 100 μ L of Stop Solution to each well and read OD at 450 nm immediately using the microplate reader.

For Human IgM Assay

1. Wash the 96-Well COVID-19 Spike Protein Coated Microplate with 1x PBST for 30 second at a time, with gentle shaking on the shaker.
2. Add 200 μ L of Blocking Solution and incubate for 1 hour at room temperature.
3. Wash 2 times with 200 μ L of 1x PBST for 30 second at a time on the shaker.

4. Add 100 μL of Human Serum Diluent to the corresponding wells, cover with parafilm and incubate for 1 hour at room temperature.
5. Wash 5 times with 200 μL of 1x PBST for 30 second at a time on the shaker.
6. Add 80 μL of Anti-human **IgM** antibody (HRP) Diluent to corresponding wells and incubate for 1 hour at room temperature with gentle shaking on the shaker.
7. Wash 5 times with 200 μL of 1x PBST for 30 second at a time on the shaker.
8. Add 100 μL of TMB Reagent to each well and incubate for 6 minutes at room temperature in the dark with gentle shaking on the shaker. TMB Reagent should be return to room temperature before use *Note: TMB Reagent is a light-sensitive reagent. Keep away from light.*
9. Add 100 μL of Stop Solution to each well and read OD at 450 nm immediately using the microplate reader.