INTENDED USE

Camel Anti-Middle Eastern Respiratory Syndrome Virus spike protein S1 domain (**Anti-MERS-S1 IgG)** ELISA Kit is an indirect ELISA suitable for quantifying IgG antibody activity specific for MERS-S1 serum, plasma or other qualified biological samples from vaccinated, immunized and/or infected hosts.

This immunoassay is suitable for:

- Determining **immune status** relative to non-immune controls;
- Assessing efficacy of **vaccines**, including dosage, adjuvantcy, route of immunization and timing;
- Qualifying and standardizing vaccine batches & protocols.

The assay is for research use only (RUO) and is not intended nor therapeutic uses.

GENERAL INFORMATION

MERS is a viral respiratory infection caused by the newly identified MERS-coronavirus (MERS-CoV), a betacoronavirus derived from bats. MERS can range from asymptomatic disease to severe pneumonia leading to the acute respiratory distress syndrome. As of Jan 2015, MERS caused more than 900 been reported in several Middle Eastern countries, Bangladesh, the United Kingdom, and the United States. Early research suggested the virus is related to one found in the bats and in dromedary camels, as 90-100% camels have antibodies to the MERS-CoV spike protein. Human or animals diagnostic serology is based upon PCR or ELISA or antibody neutralization tests. There are no vaccines available for MERS.

MERS-CoV, a +RNA viruses from Betacoronavirus lineage C, is more closely related to the bat coronaviruses HKU4 and HKU5 (lineage 2C, ~90% identity) than it is to SARS-CoV (lineage 2B). Serologic analysis of CoVs is challenging because of cross-reactivity between CoVs infecting the same host and the broad distribution of CoVs in diverse mammalian species. Many small animals (mice, hamsters and ferrets) who lack the functional MERS-CoV receptor (DPP4) and are not susceptible to infection.

MERS produces structural proteins (Spike, S; Envelope (E), Membrane (M), and Nucleocapsid protein (NP). S protein (1353-aa) has 2 well defined domains: **S1** (1-751aa) and S2 (752-1353aa). During viral entry, the S protein is cleaved into S1 and S2 subunits by host cell derived proteases. S1 subunit mediates virus binding to cells expressing DPP4 through its receptor-binding domain (RBD, 367-606 aa) region and an S2 subunit that mediates virus-cell membrane fusion. A truncated RBD domain (377-588) protein binds efficiently to DPP4. NP protein is required for RNA synthesis, and has RNA chaperone activity. The presence of MERS viral antibodies (N, E and S, and S1) have been used to detect the infected animal or humans.

PRINCIPLE OF THE TEST

The Anti-MERS S1 Ig's (IgA/IgG/IgM) ELISA kits are based on the binding of antibodies in samples to the purified MERS antigen immobilized on the microwells. Bound antibody is detected by anti-IgG or IgM-HRP conjugate (species specific). After a washing step, chromogenic substrate (TMB) is added and color (blue) developed. Stop Solution is added to terminate the reaction, and Absorbance (yellow color) is then measured using an ELISA reader at 450nm, which is directly proportional to the amount of antibody present in the sample. The presence of antibody (IgA/IgG/IgM) in samples is determined relative to anti-MERS S1 Ig's Calibrators and Controls.

ADI has cloned and expressed various MERS recombinant proteins and made antibodies. Preliminary data suggest that anti-S1 or S1-RBD and anti-NP antibody ELISA kits may provide the most specific analyses of MERS-Cov infection in humans and animals.

KIT CONTENTS

The microtiter well plate and all other reagents, if unopened, are stable at 2-8^{o}C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Component	Preparation Instructions
Wash Solution Concentrate (50x) Cat. #WB-50, 10 ml	Dilute the entire volume 10ml + 490 ml with distilled or deionized water into a clean stock bottle. Label as ***1X* Wash Solution** and store at 4^{o}C for long term and ambient temp. for short term.
Sample Diluent Concentrate (20x) Cat. No. SD-20TG, 10 ml (green solution)	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as 1**X Sample Diluent** and store at 2-8^{o}C until the kit lot expires or is used up.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
MERS-S1 Coated Strip Plate	402201	8-well strips (12)	Coated with MERS-S1, and post-coated with stabilizers.
Anti-MERS S1 IgG Calibrators			
1 U/ml **3 U/ml** **10 U/ml** **30 U/ml**	402212A 402212B 402212C 402212D	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-MERS S1; in buffer with antimicrobial.
Anti-MERS S1 IgG positive Control	402213-PC	0.65 ml (green cap)	Camel serum with anti-S1 IgG reactivity; **Net OD > 0.6**
Anti-camel IgG-HRP Conjugate	GCL1-1	12 ml	(pink solution) provide in buffer with detergents and antimicrobial
TMB Substrate	80091	12 ml	Chromogenic substrate for HRP containing TMB and peroxide.
Stop Solution	80101	12 ml	Dilute sulfuric acid.

Materials Required But Not Provided:

- Pipettors and pipettes that deliver 100ul and 1-10ml.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Camel IgG HRP Concentrate.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength and ELISA plate washer

ASSAY DESIGN AND SET-UP

Sample Collection and Handling

Serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For **serum**, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage.

Sample Dilution & Antibody Stability

Prepare an initial sample dilution of 1:10 (20 ul sample into 180 ul of **1X Sample Diluent)** in order to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for months when stored refrigerated or frozen. Additional testing dilution of 1:100, 1:200, 1:500 or 1:1000 should be prepared from 1:10 stock in sample diluent (green solution) to reduce non-specific background.

Example: Prepare 1:200 test dilution
Dilute 1:10 stock another 1:20 (20 ul of 1:10 and 180 ul of 1x diluent; final sample dilution 1:200). Use test dilution that provides low assay background and good discrimination of specific signal. Sample dilutions should be tested in the range of 1:50-1:1000 before testing al. samples. Do not store final test dilutions.

Assay Design

Review Interpretation of Results (p5-7) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be lower than the **1 U/ml Calibrator**. This is usually 1:200 or greater dilution for Camel serum with normal levels of IgG and IgG.
- Run the Anti-MERS-S1 IgG Positive Control; net OD > **0.5**.
- Run a Sample Diluent **Blank**. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required. Blank OD should be <0.3.
- Run a set of **Calibrators**, which validate that the assay was performed to specifications: **30 U/ml** should give a high signal (>1.5 OD); **1 U/ml** should give a low signal which can be used to discriminate at the Positive/Negative threshold (see Interpretation of Results, p. 5).

Plate Set-up

Bring all reagents to room temperature (18-30^{o} C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run. Duplicates are recommended, including 8 Calibrator wells and 2 wells for each sample control to be assayed.
- Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul – 60 min; 4 washes]

- Add 100ul of calibrators, samples and controls each to pre-determined wells.
- Tap the plate gently to mix reagents and incubate for 60 minutes.
- Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul – 30 min; 5 washes]

- Add 100ul of diluted Anti-Camel IgG HRP to each well.
- Incubate for 30 minutes.
- Wash wells 5 times as in step 2.

3. Substrate Incubation [100ul – 15 min]

- Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.
- Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

- Add 100ul of Stop Solution to each well.
- Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

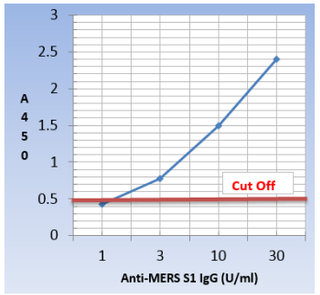
- Use any commercially available microplate reader capable of reading at 450nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
- Read absorbance of the entire plate at 450nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630nm to normalize well background.

PRECAUTIONS AND SAFETY INSTRUCTIONS

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water. MSDS for TMB, sulfuric acid and BND can be requested

WORKSHEET OF A TYPICAL ASSAY

Wells	Stds/samples	Mean A450	Net A450
A1/2	Blanks (sample diluent)	0.100	-
B1/2	Calibrator A (1 u/ml)	0.55	0.43
C1/2	Calibrator B (3 u/ml)	0.873	0.773
D1/2	Calibrator C (10 u/ml)	1.66	1.56
E1/2	Calibrator D (30 u/ml)	2.58	2.48
G1/G2	**Negative sample**	0.45	0.35
H1/H2	**Positive Control**	1.7	1.6



11_ADI-ELISA

The above graphs is for demonstration purpose only. Actual values may differ lot to lot but be close to the above illustration. Use the lot specific curve for calculating the sample values.

INTERPRETATION OF RESULTS

Qualitative Results

1. Calculate the average A450 of the blanks, -ve and positive control.
2. Subtract the average blanks values from the average values of the controls and samples.
3. **Arbitrary Cut-off values**: Add 0.10 to the negative control values of negative control values supplied with the kit or User supplied – ve control (if available). A redline has been drawn in the above graphs to represent the '**Cut-off values**"
4. **Sample values at or below the cut**-off values can be treated as –ve and above it are positive.

Examples: Net Average negative control values =0.405
Cut-Off: Add 0.100 (0.405+0.100) =0.505
Negative samples <0.505
Positive samples >0.505

Arbitrary cut-off values as used in the example here are based upon our regional sample values. We strongly recommend that users set-up their own negative and cut-off values based upon samples from the region. **No single cut-off value may truly represent samples from all over the world.

Quantitative Results

1. Calculate the average A450 of the blanks, calibrators controls, and samples
2. Subtract the average blanks values from the average values of the calibrator and samples
3. Plot the net average A450 values of calibrators against the concentration (u/ml).
4. We recommend using point-to-point graphs.
5. Calculate the unknown sample values from the graph.
6. Multiply the values by the dilution factor of the samples.

The **sensitivity** of the assay to detect anti-MERS-NP IgG, from either natural infection or vaccination, is controlled so that the **1 U/ml Calibrator** represents a threshold OD for most true positives in camel serum **diluted 1:200 or greater**. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of an anti-MERS-S1 antibody, derived from MERS vaccination, shows the OD range of the assay; high value indicates optimal sensitivity of the assay. **1 U/ml**: a 'Cut-off' line has been drawn to indicate a threshold distinguishing between **Positive/Negative**. This is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

INTERPRETATION OF RESULTS

1. **Positives** may be due to prior encounter with the virus or vaccination immunization.

2. The **sensitivity** of the assay may be adjusted by changing the sample dilutions: a) increase dilution >1:100 (e.g., 1/500) to lower the signals of borderline positives to negative; b) decrease dilution (e.g., 1/50) to convert borderline samples to positive. With the latter, the values of negatives may increase, so an alternative "Cut-off" should be considered using known negatives (Page 5).

QUALITY CONTROL

1. When using the test for the first time, we recommend that the user run only the controls and standards to get familiar with the kit and proper execution of the entire procedure.

2. **Blank values must not exceed >0.3** as it will indicate general failure to wash the plates properly. In case, of high blanks and overall high values in all wells, repeat the test using just 1 strip until proper blanks and reference values are obtained

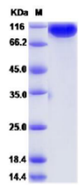
indicate general failure to wash the plates properly. In case, of high blanks and overall high values in all wells, repeat the test using just 1 strip until proper blanks and reference values are obtained.

3. **Calibrators, negative and positive controls must be within the range** or values specified in the manual. Some variations are acceptable (5-20%) of the value due to variations in incubation temp and time and the running efficiency of the protocol.

4. Users must always run internal reference controls

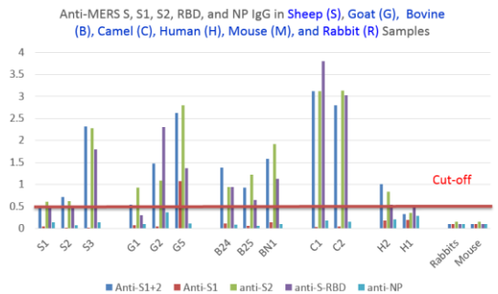
PRODUCT SPECIFICATIONS

Antigen Specificity



Recombinant (HEK), purified (95%, ~79 kda), full length MERS-S1 protein MERS-CoV) ([Human betacoronavirus 2c EMC/2012]) is used as antigen. Human MERS-S1 is conserved in Bat HKU-4 (62%), and Bat HKU-5 (58%). No significant conservation in SARS CoV or Bovine Coronavirus or (gamma lineage of CoV). It is not known if the above species S1 antibodies are present in animals or humans and if they are cross-reactive.

MERS Antibodies in Sheep (S) Goat (G), Bovine (B), Camel (C), Human (H), Rabbit, and Mouse samples – Samples tested at 1:100-:500 using ADI's MERS-S, MERS-S1, MERS-S2, MERS-RBD, and MERS-NP IgG ELISA kits for various species.



Antibodies to various MERS protein, Spike (full length S), S1, S2, Spike-RBD domains, and NP IgG showed great variance. Antibodies to the whole spike protein or S2 domain were more common than the anti-S1 or anti-NP. There are no published reports regarding the specificity of the MERS antibodies. It is possible that Spike domain S2 has the epitopes that are common with other related virus. Therefore, we recommend that users test various ELISA kits under controlled conditions to draw any conclusion about the MERS protein antibodies and MERS infection.

Instruction Manual No. RV-402210-1

Recombivirus Camel Anti-Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Spike Protein S1 domain (MERS-S1) IgG ELISA kit

Cat # RV-402210-1, 96 tests

For Quantitation of Anti-MERS-S1 IgG in Camel Serum or Plasma

For research use only, not for diagnostic or therapeutic use.

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