INTENDED USE

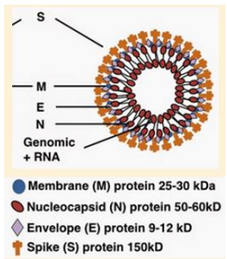
Camel Anti-MERS-S2 IgG ELISA Kit is an indirect ELISA suitable for quantifying IgG antibody activity for MERS-S2 protein in camel serum, plasma or other qualified biological samples from vaccinated, immunized and/or infected hosts.

This immunoassay is suitable for:

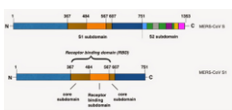
- Determining **immune status** relative to non-immune controls;
- Assessing efficacy of **vaccines**, including dosage, adjuvantcy, route of immunization and timing;
- Qualifying and standardizing vaccine batches & protocols.

Recombivirus series of ELISA kits use recombinant purified antigens; no virus or viral proteins are used in the kit. The assay is for research use only, not for diagnostic use.

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. 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. There are **no vaccines** available for MERS. 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) 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. NP protein is required forRNA synthesis, and has RNA chaperone activity. The presence of MERS viral antibodies (N, E and S, and S1) have been used to detect MERS infection.

PRINCIPLE OF THE TEST

The Anti-MERS S2 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 S2 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^{0}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 (100x) Cat. #WB-100, 10 ml	Dilute the entire volume 10ml + 990 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-20T, 10ml	Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as **1X Sample Diluent** and store at 2-8^{o}C until the kit lot expires or is used up.
Anti-Camelid IgG- HRP Conjugate Concentrate (100x) Part: H-rLaG.311, 0.15ml	Peroxidase conjugated anti-camelid IgG in buffer with detergents and antimicrobial. Dilute fresh as needed; 10ul of concentrate to 1ml of **1X Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8^{o} C storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
MERS-S2 Coated Strip Plate	402301	8-well strips (12)	Coated with MERS-S2, and post-coated with stabilizers.
Anti-MERS S2 Calibrators			
1 U/ml 2.5 U/ml 5 U/ml 10 U/ml	402302A 402302B 402302C 402302D	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-MERS S2; in buffer with antimicrobial.
AntiMERS S2 Positive Control	402302-PC	0.65 ml	Serum with anti-S2 reactivity; **Net OD > 0.6**
Low NSB Sample Diluent	TBTm	30 ml **(Green Soln)**	Buffer with protein, detergents and antimicrobial as stabilizers. Use as is for sample dilution
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.
- 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 Low NSB diluent (green solution) to reduce non-specific binding.

Example: Prepare 1:200 test dilution
Dilute 1:10 stock another 1:20 (15 ul of 1:10 and 285 ul of Low NSB diluent (green soln); 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 1:200-1:1000 before testing all 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 1:100 or greater dilution for camel serum with normal levels of IgG and IgM.
- Run the Anti-MERS-S2 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: **10 U/ml** should give a high signal (>1.5 OD); **1 U/ml** should give a low signal that 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 8Calibrator 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 1x sample diluent (blank), 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-Camelid 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 or obtained from the ADI website:http://4adi.com/commerce/info/showpage.jsp?page_id=1060&category_id=2430&visit=10

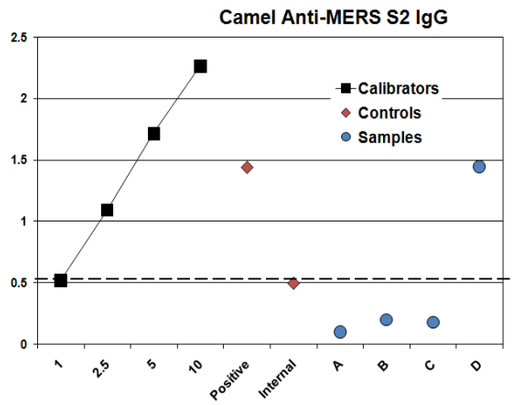
INTERPRETATION OF RESULTS

A. Antibody Activity Threshold Index

Compare Samples to **1 U/ml Calibrator** or **Internal Control**

=Positive/Negative Cut-off.

Example:



Results

The **sensitivity** of the assay to detect anti-MERS-S2 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 to 1:100 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of antiserum from anti-MERS-S2 immunization, 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.

Positive Control – an anti-MERS-S2 serum; net OD >**0.5**. This Control can be used to normalize between-assay variation.

Internal Control – a true positive from an immune individual that represents the investigator's experience in distinguishing low positive from negative samples (not in kit). This should be run in each assay to supplement the 1 U/ml Calibrator for Positive/Negative discrimination purposes.

Samples A,B,C,D – 3 samples (1:100) (A, B, C) are negative: below the threshold; 1 sample (D) is positive: clearly above the threshold.

The 1 U/ml Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative:

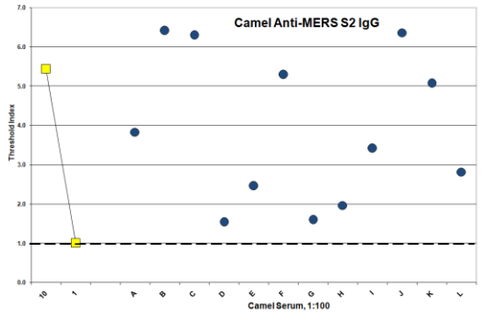
- Divide each Sample net OD by the 1 U/ml Calibrator net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 is **Negative** for antibody.

INTERPRETATION OF RESULTS (cont)

Example:

Camel Serum IgG

A panel of camel sera of unknown history was tested for anti-MERS-S2 IgG (1:100 dilution). **Threshold Index** was calculated using the **1** U/ml **Calibrator.**



Results

Anti-MERS-S2 IgG: all sera (12) were Positive, above the **1.0** Threshold Index, at 1:100 dilutions.

Notes:
1. **Positives** may be due to prior encounter with the virus or non-MERS antibodies directed against common epitopes, or may be an aspect of the innate immune repertoire.
2. The **sensitivity** of the assay may be adjusted by changing the sample dilutions: a) increase dilution (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 threshold should be considered using known negatives to develop a **Positive Index (**see below) or by using an **Internal Control** (Page 5).
3. Samples with a **Theshold Index** above **5.0** should be tittered using a dilution curve as in Method C for best accuracy.

B. Positive Index

Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a **Positive Index**. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Non-immune samples = **Positive Index**.

2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 is **Negative** for antibody.

A sample value would be **Positive** if significantly above the value of the pre-immune serum sample or a suitably determined non-immune panel or pool of samples, tested at the same sample dilution.

Method C. Titers from Sample Dilution Curves

The titer of elevated antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method. Best precision can be obtained using the following guidelines:

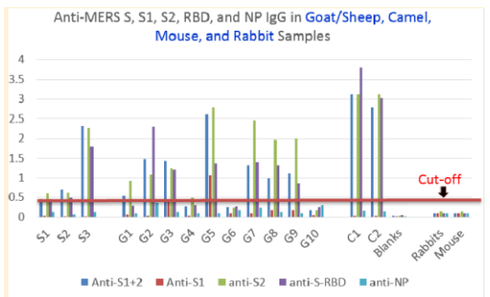
2. Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.
3. Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.
4. A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data.
5. The Calibrator values can be used to normalize inter-assay values.
6. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis) corresponding to the OD of the selected Index

PRODUCT SPECIFICATIONS

Antigen Specificity

Recombinant (sf9), MERS-S2 (95%, ~66 kda, full length) MERS-S2 protein) (Human betacoronavirus 2c EMC/2012) is used as antigen. MERS-CoV spike protein 2 sequence is conserved in the bat coronaviruses HKU4 (73%) and HKU5 (70%) and BtCoV strains (73%).. It is not known if the above species S2 antibodies or proteins are present in animals or humans and if they are cross-reactive.

MERS Antibodies in Sheep (S) Goat (G), Rabbit, mouse, and Camels (C)–All samples tested at 1:100 using ADI's MERS-S, MERS-1, MERS-S2 and MERS-NP ELISA kits for various species.



Reactivity to various MERS protein, **Spike** (full length) or **S1** and **S2** domains, **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-S1 RBD domain or NP.

Instruction Manual No. RV-402310-1

Recombivirus Camel Anti-Middle East Respiratory Syndrome Coronavirus (MERS-CoV) Spike Protein S2 domain (MERS-S2) IgG ELISA Kit

Cat # RV-402310-1, 96 tests

For Quantitation of Anti-MERS-S2 IgG in Camelid Serum or Plasma or other biological fluids

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

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