

## INTENDED USE

**Bat Anti-MERS-S1 IgG ELISA Kit** is an indirect ELISA suitable for quantifying IgG antibody activity for MERS-S1 protein in bat serum, plasma or other qualified biological samples from vaccinated, immunized and/or infected hosts.

This immunoassay is suitable for:

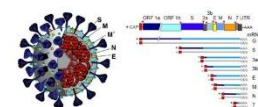
- Determining **immune status** relative to non-immune controls;
- Assessing efficacy of **vaccines**, including dosage, adjuvantcy, route of immunization and timing;
- Qualifying and standardizing vaccine batches & protocols.

Recombinant 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. MERS is related to the virus found in the bats and in dromedary camels. Approx. 90-100% Bats may have antibodies to the MERS-S1 protein. There are no vaccines available for MERS.

CORONAVIRUS STRUCTURE AND GENE EXPRESSION

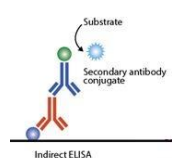


MERS-CoV, is more closely related to the bat coronaviruses HKU4 and HKU5 (lineage 2C, ~90% identity) than it is to SARS-CoV (lineage 2B). Serologic analysis of CoVs is challenging because of cross-reactivity between CoVs

infecting the same host and the broad distribution of CoVs in diverse mammalian species.

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 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. Human or animals diagnostic serology is based upon PCR or ELISA or antibody neutralization tests.

## 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 with 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°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                                                                                                                                                                                                                                                |
|----------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Wash Solution Concentrate (100x)</b><br>Cat. #WB-100, 10 ml                   | Dilute the entire volume 10ml + 990 ml with distilled or deionized water into a clean stock bottle. Label as <b>1X Wash Solution</b> and store at 4°C for long term and ambient temperature for short term.                                                             |
| <b>Sample Diluent Concentrate (20x)</b><br>Cat. No. SD-20T, 10ml                 | Dilute the entire volume, 10ml + 190ml with distilled or deionized water into a clean stock bottle. Label as <b>1X Sample Diluent (WSD)</b> and store at 2-8°C until the kit lot expires or is used up.                                                                 |
| <b>Anti-Bat IgG- HRP Conjugate Concentrate (100x)</b><br>Part: H-BtG.211, 0.15ml | Peroxidase conjugated anti-bat IgG in buffer with detergents and antimicrobial. Dilute fresh as needed; 10ul of concentrate to 1ml of <b>1X Sample Diluent</b> is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8° C storage. |

**Ready For Use:** Store as indicated on labels.

| Component                                | Part      | Amt                          | Contents                                                                                        |
|------------------------------------------|-----------|------------------------------|-------------------------------------------------------------------------------------------------|
| <b>MERS-S1 Coated Strip Plate</b>        | 402201    | 8-well strips (12)           | Coated with MERS-S1, and post-coated with stabilizers.                                          |
| <b>Bat Anti-MERS S1 Calibrators</b>      |           |                              |                                                                                                 |
| 1 U/ml                                   | 402222B   | 0.65 ml                      | Four (4) vials, each containing anti-MERS S1; in buffer with antimicrobial.                     |
| 2.5 U/ml                                 | 402222C   | 0.65 ml                      |                                                                                                 |
| 5 U/ml                                   | 402222D   | 0.65 ml                      |                                                                                                 |
| 10 U/ml                                  | 402222E   | 0.65 ml                      |                                                                                                 |
| <b>Bat Anti-MERS-S1 Positive Control</b> | 402222-PC | 0.65 ml                      | Serum with anti-S1 reactivity;<br><b>Net OD &gt; 0.6</b>                                        |
| <b>Low NSB Sample Diluent</b>            | TBTm      | 30 ml<br><b>(Green Soln)</b> | Buffer with protein, detergents and antimicrobial as stabilizers. Use as is for sample dilution |
| <b>TMB Substrate</b>                     | 80091     | 12 ml                        | Chromogenic substrate for HRP containing TMB and peroxide.                                      |
| <b>Stop Solution</b>                     | 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-Bat 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 1:5 (20 ul sample into 80 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 1:25 or greater should be prepared from 1:5 stock in Low NSB diluent (green solution) to reduce non-specific binding.

**Example:** Prepare 1:50 test dilution

Dilute samples 1:5 (10 ul sample in 40 ul 1X WSD)  
Dilute 1:5 stock another 1:10 (25 ul of 1:5 and 225 ul of Low NSB diluent (green soln); final sample dilution 1:50). Use test dilutions that provide low assay background and good discrimination of specific signal. Sample dilutions should be tested in the range of 1:50-1:1000 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 1:50 or greater dilution for bat serum with normal levels of IgG and IgM.
- Run the Bat Anti-MERS-S1 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: **20 U/ml** should give a high signal (**>1.5 OD**); **1 U/ml** should give a low signal which may 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° 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<sup>st</sup> 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<sup>nd</sup> Incubation** [100ul – 30 min; 5 washes]
  - Add 100ul of diluted Anti-Bat IgG HRP to each well.
  - Incubate for 30 minutes.
  - Wash wells 5 times as in step 2.
- 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).
- 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.
- 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/objects/catalog/product/extras/ELISA-Kit-SDS-MSDS-Set-1.pdf>

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

Cat # RV-402220-1, 96 tests

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

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



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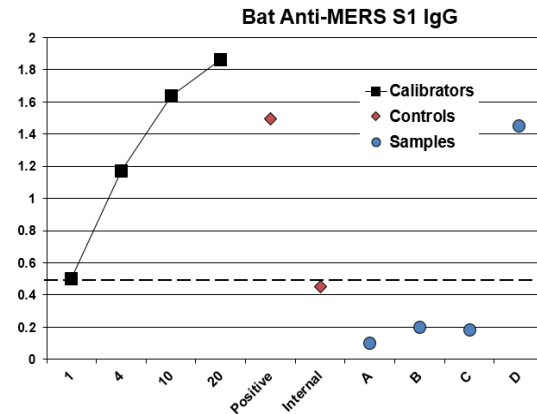
### INTERPRETATION OF RESULTS

#### 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-S1 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 bat serum diluted to 1:50 - 100 or greater. Visual inspection of the data in the above graph shows the following:

**Calibrators** – dilution curve of antiserum from anti-MERS-S1 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-S1 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:

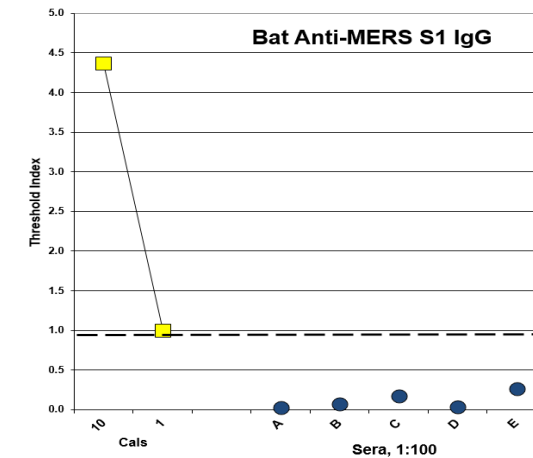
❖ Divide each Sample net OD by the 1 U/ml Calibrator net OD. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 is **Negative** for antibody.

### INTERPRETATION OF RESULTS (continued)

#### Example:

#### Bat Serum IgG

A panel of sera from native bats was tested for anti-MERS-S1 IgG (1:100 dilution). **Threshold Index** was calculated using the 1 U/ml Calibrator.



#### Results

**Anti-MERS-S1 IgG:** all sera were negative (at or below the 1.0 Threshold Index). The low values suggest a more concentrated serum (e.g., 1: 50) may be used to detect low positive samples.

#### Notes:

- Positives** may be due to prior encounter with the virus or anti-MERS-S1 immunization.
- The **sensitivity** of the assay may be adjusted by changing the sample dilutions: a) increase dilution (e.g., 1:200) 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 use an **Internal Control** (Page 5).

#### 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:

- Calculate the net OD mean + 2 SD of the Control/Non-immune samples = **Positive Index**.
- Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of **Positive** Antibody Activity; below 1.0 are **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:

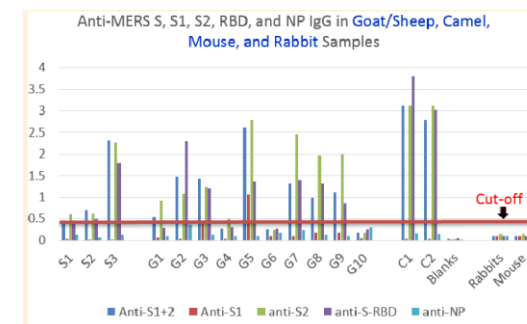
- Use an OD value Index in the mid-range of the assay (2.0 – 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.
- Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.
- 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.
- The Calibrator values can be used to normalize inter-assay values.
- 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 (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 (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), 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.

| ELISA Kit Components                 | Amount             | Part         |
|--------------------------------------|--------------------|--------------|
| MERS-S1 Coated Microwell Strip Plate | 8-well strips (12) | 402201       |
| Bat Anti-MERS-S1 Positive Control    | 0.65 ml            | 402222PC     |
| Bat Anti-MERS-S1 Calibrator          | 1 U/ml             | 402202B      |
| Bat Anti-MERS-S1 Calibrator          | 4 U/ml             | 402222C      |
| Bat Anti-MERS-S1 Calibrator          | 10 U/ml            | 402222D      |
| Bat Anti-MERS-S1 Calibrator          | 20 U/ml            | 402222E      |
| Anti-Bat IgG HRP Conjugate (100X)    | 0.15 ml            | H-BTG.211    |
| Sample Diluent (20X)                 | 10 ml              | SD20T        |
| Low NSB Sample Diluent               | 30 ml              | TBTm         |
| Wash Solution Concentrate (100X)     | 10 ml              | WB-100       |
| TMB Substrate                        | 12 ml              | 80091        |
| Stop Solution                        | 12 ml              | 80101        |
| Product Manual                       | 1 ea               | M-RV402220-1 |