INTENDED USE

The Human Anti-E. coli proteins IgG ELISA Kit is an indirect ELISA suitable for detecting and quantifying antibody activity (IgG) specific for E. coli (*Escherichia coli)* Host Cell Proteins (HCP) in serum, plasma or other biological fluids. Antibodies may occur from either natural E. coli encounter or from immunization with recombinant proteins expressed in E. coli. This kit is for research use only (RUO), not for diagnostic purposes.

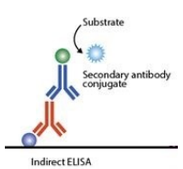
GENERAL INFORMATION

A large number of genes have been cloned and expressed in various host cells (E. coli, yeast, baculovirus, NSO, Sp2/0, HEK, CHO cells). The translated recombinant proteins may remain within the cell, requiring host cell disruption for release, and/or may be secreted into the culture medium. The target recombinant proteins (recProtein) would then be purified from unwanted host cell protein (HCP), often with the aid of a tag (e.g., His, GST, MBP). During the production of recProteins, host cells die and decompose; thus, regardless of whether the recombinant product is obtained from extracellular medium or after disrupting the host cell, the entire repertoire of host cell proteins present as potential contaminants in downstream purification and processing of the recProtein product.

The presence of residual E. coli HCPs in purified recombinant proteins and its use as antigens must be carefully studied before using it for the detection of human antibodies. SARS-NP expressed in E. coli has resulted in false positive due to the detection of E. coli antibodies (refs 1). E. coli proteins (LPS, outer membrane proteins or residual HCPs) are highly immunogenic in humans and animals. Most humans and animals have exposure to E. coli and have varying levels of circulating antibodies. Therefore, human samples must be tested before and after exposure of E. coli containing proteins or other materials.

HCP in the processed recombinant may or may not be detectable by SDS-PAGE, ELISA, etc, which could have limited sensitivity or specificity for the particular E. coli protein(s) present. Immunization with preparations containing undetected HCP, however, often generate anti-HCP antibodies along with the specific anti-recProtein antibodies. Thus, the immunization process can represent a more sensitive method than others for disclosing low level HCP contamination. Alternatively, the unwanted anti-HCP activity may obscure and/or confuse the interpretation of immunoassays designed to characterize and utilize the anti-recProtein activity.

PRINCIPLE OF THE TEST



The Human Anti-E coli HCP IgG ELISA kit is based on the binding of antibody in samples to E coli HCP antigen immobilized on the microwells, and bound antibody is detected by anti-Human IgG-specific antibody conjugated to HRP conjugate. After a washing step, chromogenic substrate (TMB) is added and color (blue), which is directly proportional to the amount of antibody present in the sample. Stop Solution is added (converts blue to yellow color), and A450nm is then measured using an ELISA reader. The activity of antibody in samples is calculated relative to supplied calibrators.

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 (100x) Cat. No. WB-100, 10ml	Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as **Working Wash Solution** and store at ambient temperature until kit is used entirely.
Sample Diluent Concentrate (20x) Cat. No. SD-20T, 10ml	Dilute 0.5ml + 9.5ml with distilled or deionized water as needed for HRP Conjugate and Sample Dilution. Label as **Working Sample Diluent** and store at 2-8ºC until the kit lot expires or is used up.
Anti-Human IgG - HRP Conjugate Concentrate (100x) Part No. H-HuG.211, 0.15ml	Peroxidase conjugated anti-Human IgG in buffer with protein, detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of **Working Sample Diluent** is sufficient for 1 8-well strip. Use within the working day and discard. Return 100X to 2-8ºC storage.

Ready For Use: Store as indicated on labels.

Component	Part	Amt	Contents
E coli HCP Antigen Microwell Strip Plate	500-101	8-well strips (12)	Coated with E coli HCP antigen, and post-coated with stabilizers.
Human Anti-E coli HCP Calibrators			
1 U/ml 2.5 U/ml 5 U/ml 10 U/ml	500-112B 500-112C 500-112D 500-112E	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing anti-E coli HCP levels in arbitrary activity Units; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
Human Anti-E coli Positive Control	500-112PC	0.65 ml	Human serum with anti-E coli HCP activity. Net OD > **0.5**
Low NSB Sample Diluent	TBTm	30 ml	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. A multi-channel pipettor is recommended.
- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Human IgG HRP Concentrate.
- Stock bottle to store diluted Wash Solution; 200ml to 1L.
- Distilled or deionized water to dilute reagent concentrates.
- Microwell plate reader at 450 nm wavelength.

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.

Caution: Human serum and other bodily fluids may contain infectious material. Always wear gloves when handling human samples, including the standards and controls (which have been tested non-reactive for HbsAg and Anti-HIV), and dispose of these samples and containers as biohazard waste.

Antibody Stability
Initial dilution of serum into **Working Sample Diluent** is recommended to stabilize antibody activity. This enhances reproducible sampling, and stabilizes the antibody activity for years, stored refrigerated or frozen. Further dilution into **Low NSB Sample Diluent**, which provides the lowest assay background, should be at least 5 times the initial dilution and performed the same day as the assay.

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 1:500 or greater dilution for human serum with normal levels of IgG and IgM.
- Run the Human Anti-ZEBOV GP 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: **10 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 (18-30º 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-Human 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 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: Sample Diluent and anti-Protein G-HRP contain Proclin 300 (0.05%, v/v). http://4adi.com/objects/catalog/product/extras/ELISA-Kit-SDS-MSDS-Set-1.pdf

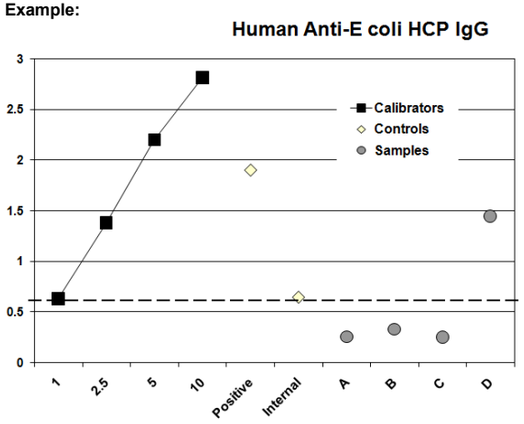
INTERPRETATION OF RESULTS

Method 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-E coli HCP 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 human serum diluted to 1:500 or greater. Visual inspection of the data in the above graph shows the following:

Calibrators – dilution curve of an anti- E coli HCP antibody, derived from E coli antigens 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**. The is not a clear-cut threshold, rather a low OD area that could represent either low positives or high background negatives.

Positive Control – a human antibody showing natural reactivity to E coli antigen; net OD > **0.5**. This Control can be used to normalize between-assay variation.

Internal Control – a true positive from an immunized human that represents the lab's experience in distinguishing low positive from negative samples. 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 (A, B, C) are negative: below the threshold; 1 sample (D) is positive: clearly above the threshold.

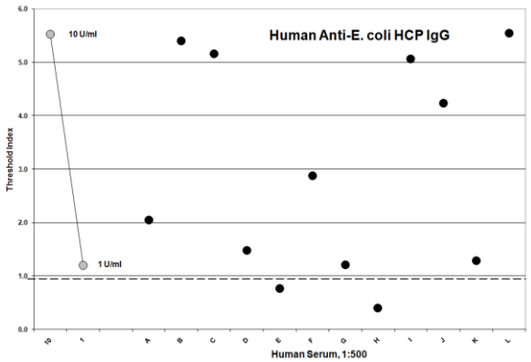
The 1 U/ml Calibrator can be used to calculate a **Threshold Index** that numerically discriminates Positive/Negative:

- 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 are **Negative** for antibody.

CALCULATION OF RESULTS (continued)

Assay Performance

Human Serum/Plasma IgG
A panel of human serum/plasma of unknown history were tested for anti-E coli HCP (1:500 dilution). **Threshold Index** was calculated using the **1** U/ml **Cal**.



Results

Seven (7) sera showed significant **Positive** titer (Index > 2); 4 sera were modestly elevated or borderline (Index >1<2); 1 serum was below the Threshold Index, indicating very low to **Negative** anti-E coli titer.

Notes:

1. **Positives** may be due to prior encounter with the bacteria or non-E coli proteins with common epitopes, or may be an aspect of the innate immune repertoire.
2. Samples with **Positive Threshold Indexes** above **5.0** should be diluted as in Method C to more accurately assess titers.
3. The sensitivity of the assay may be increased to perhaps convert a borderline sample to a positive by using a lower dilution of the sample, e.g., 1/50. The values of negatives may increase, so an alternative threshold should be established using known negatives to develop a **Positive Index** (page 6), or by using known **Internal Controls** as discriminator for a **Threshold Control** (instead of the kit 1 U/ml Calibrator Control)

Method 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 are **Negative** for antibody.

CALCULATION OF RESULTS (continued)

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.

This calculation also **quantifies** the positive Antibody Activity level, assigning a higher value to samples with higher Antibody Activity, and vice versa.

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:

1. 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.
2. 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.
3. 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.
4. The Positive and Sensitivity Control values can be used to normalize inter-assay values.

Calculations
1. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
2. 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
   = **IgG Antibody Activity Units**

ASSAY LIMITS

A multitude of E. coli proteins are represented as the antigen coating on the microwell plate, with the individual proteins coating at different high to low concentrations. For this reason, anti-E. coli HCP antibodies directed against different proteins may vary in the sensitivity for which they are detected; some anti-HCP antibodies may not be detected above background = **False Negative**. False negatives in this assay may be positive in another assay system where the specific antigen is better represented.

PRODUCT SPECIFICATIONS

Specificity
The plate is coated with recombinant WNV Non-structural Protein 1 protein (E.coli, his-tag, ~42 kDa), which has been shown to be antigenic relative to WNV challenge. Therefore, this kit will only detect antibodies to the E coli HCP protein, and not to prM or capsid or other proteins. The anti-Human IgG HRP conjugate specifically detects IgG, and does not react with IgM, IgA or IgE class antibodies above background.

Instruction Manual No. M-500-110-ECP

Human Anti - E coli HCP (Host Cell Proteins) IgG

ELISA Kit #. 500-110-ECP

For Quantitation of Anti-E coli HCP IgG in Human Serum or Plasma

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

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ELISA Kit Components		Amount	Part
E coli HCP Antigen Coated Microwell Strip Plate		8-well strips (12)	500-101
Human Anti-E coli Calibrator	1 U/ml	0.65 ml	500-112B
Human Anti-E coli Calibrator	2.5 U/ml	0.65 ml	500-112C
Human Anti-E coli Calibrator	5 U/ml	0.65 ml	500-112D
Human Anti-E coli Calibrator	10 U/ml	0.65 ml	500-112E
Human Anti-E coli Positive Control		0.65 ml	500-112PC
Anti-Human IgG HRP Conjugate (100X)		0.15 ml	H-HuG.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-500-110-ECP