PRODUCT SPECIFICATIONS

Specificity
Preparations containing full protein repertoires from 6 commonly used *E. coli* strains [TOP10, K12, DH5a, BL21, HB101] are used to coat the ELISA plates. Western blots of several species immunized with the preparations showed antibody reactivities towards a broad array of the proteins. These antisera also have high titers in the ELISA (see Limits of the Assay, page 7). The anti-Mouse IgG+IgA+IgM (H+L) HRP conjugate reacts with mouse IgG, IgA and IgM class antibodies that bind to E. coli proteins on the plate.

Assay Sensitivity
The E. coli HCP coating level and Anti-Mouse Ig HRP are optimized to differentiate anti-E. coli Ig from background (non-antibody) signal with mouse serum samples diluted 1:100 (see Limits of the Assay).

Calibrator Values
The Calibrators are composed of dilutions of serum from mice with anti-E. coli HCP activity. Values are assigned as arbitrary anti- E. coli activity units.

LIMITS OF THE ASSAY

- 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.

- Immunized animals that test negative for E. coli contaminant antibodies may test as positive upon additional immunizations.

- Animals that have not been immunized may have natural anti-E. coli antibodies due to prior exposure to the bacteria. It may be useful to assay a prebleed of the recProtein - immunized animal to determine if measured anti-E. coli HCP activity is natural or due to contaminated immunogen. Natural anti-E. coli activity can be removed using an E. coli HCP Absorbent, as shown in Assay Performance (page 6).

- Serum assayed at higher concentrations than 1/100 dilution may show non-specific signals above the Negative cutoff. At these lower dilutions, compare the signal with a pre-bleed of the immunized animal at the same dilution.

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Instruction Manual No. M-500-100-ECP

Mouse Anti - E. coli HCP
(Host Cell Proteins)

ELISA Kit Cat. No. 500-100-ECP

For Quantitation of Total Anti-E. coli Ig (G+A+M) in Mouse Serum

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INTENDED USE

The Mouse Anti-E. coli HCP ELISA Kit is an immunoassay suitable for detecting and quantifying total antibody activity (IgG, IgA and IgM) specific for E. coli (*Escherichia coli)* Host Cell Proteins (HCP) in serum, plasma or other biological fluids.

INTRODUCTION

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.

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.

The Anti-E. coli HCP ELISA is a sensitive, specific assay for detecting this contaminating activity and, when coupled with use of an E. coli Proteins - Agarose absorbent, can verify the removal of the anti-HCP activity. The Assay Performance example (page 6) demonstrates this HCP detection and removal strategy, using the following ADI products:

- ❑ Mouse Anti-E. coli HCP ELISA, Cat. # 500-100-ECP
- ❑ E. coli Proteins - Agarose, Cat. #EC11-G

PRINCIPLE OF THE TEST

The Mouse E. coli ELISA kit is based on the binding of Mouse anti- E. coli in samples to E. coli HCP immobilized on the microwells, and anti- E. coli Ig antibody is detected by anti-mouse IgG+IgA+IgM-specific antibody conjugated to HRP (horseradish peroxidase) enzyme. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of anti-E. coli HCP present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microwell reader. The amount of Mouse Ig in samples is determined relative to mouse anti-E. coli reference calibrators.

CALCULATION OF RESULTS (continued)

Method II. Quantitation

Anti-E. coli activity may be expressed in semi-quantitative activity units by several methods.

- ❑ Report values in OD units multiplied by the inverse of the dilution.

Typical Results:

1.06 [Sample, net OD] x **2000** [1/dilution] = **2.12 k** Activity Units

- ❑ The Calibrators are useful as internal controls to normalize between-assay signal variation, using one of the Calibrators closest to 1.0 OD as the Index.

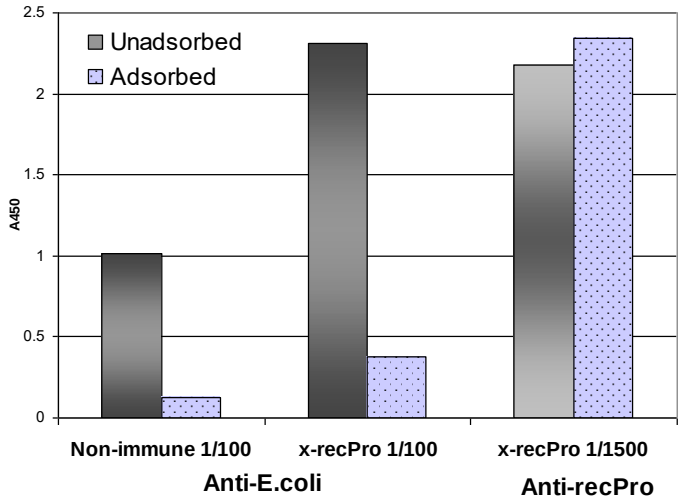
1. Calculate the mean net ODs for replicate samples and the selected Calibrator.
2. Divide each sample OD value by the Calibrator OD value, and multiply by the sample dilution = **Total Activity Units**

Typical Results:

1.06 [Sample, net OD] ÷ **1.24** [4 U/ml Calibrator, net OD]
x **2000** dilution = **1.71 k** Activity Units in serum.

ASSAY PERFORMANCE

Sera testing positive for anti-E. coli activity from a) a recombinant protein (recPro)-immunized mouse and b) a non-immunized mouse (see Limits of the Assay) were passed over an E. coli Proteins absorbent. A re-assay of anti-E. coli showed substantial removal of the 'contaminating' activity from each serum (graph). A separate assay showed the anti-recPro activity of the immunized mouse serum remained unchanged, demonstrating the specificity of the assay and usefulness of the E. coli Proteins absorbent.



CALCULATION OF RESULTS

Several data treatments may be considered, depending whether the purpose of the assay is to **Detect** or **Quantitate** anti-E. coli antibody activity.

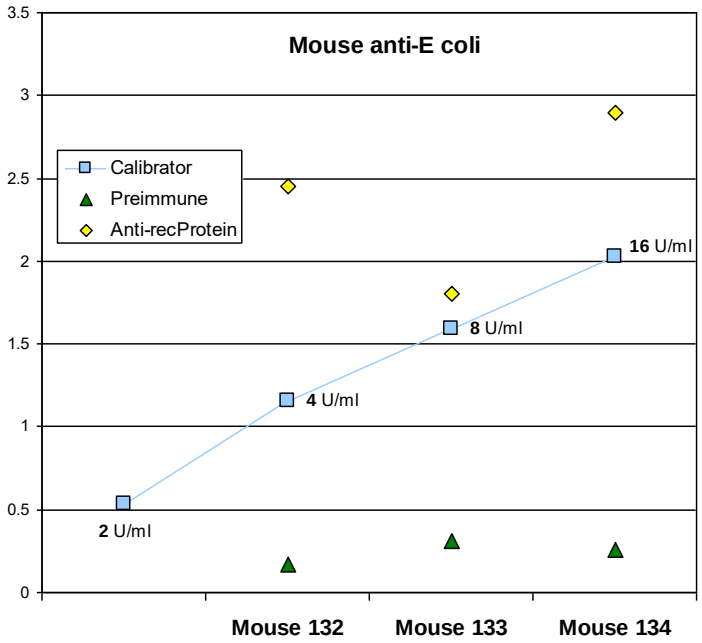
Method I. Detection

The assay is designed to differentiate anti-E. coli **Positives** from **Negatives** in mouse sera diluted at 1:100 in Sample Diluent, as follows:

1. Convert each sample and Calibrator OD value to **net OD** by subtracting the OD of the Sample Diluent blank.
2. Determine anti-E. coli activity for each as follows:

Category	Net OD
Positive	**> 0.6**
Borderline	**0.3 – 0.6**
Negative or Indeterminate (See Limits of the Assay)	**< 0.3**

Typical Results:



Sera from recombinant Protein – immunized mice (anti-recProtein) showed significant anti-E. coli activity (1:500), while pre-immune sera from the same mice tested negative (1:100). The Calibrator values confirm the range of the assay.

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	Instructions for Use
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 **Working Sample Diluent** and store at 2-8°C until the kit lot expires or is used up.
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.
Anti-Mouse Ig - HRP Conjugate Concentrate (100x) Part No. MsH-GAM, 0.15ml	Peroxidase conjugated anti-mouse IgG+IgA+IgM (H+L) 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 concentrate to 2-8° C storage.

Ready For Use: Store as indicated on labels.

Component	Part No.	Amt	Contents
E. coli Microwell Strip Plate	500-101	8-well strips (12)	Coated with E. coli HCP, and post-coated with stabilizers.
Mouse Anti-E. coli IgG Calibrators			
2 U/ml 4 U/ml 8 U/ml 16 U/ml	500-102B 500-102C 500-102D 500-102E	0.65 ml 0.65 ml 0.65 ml 0.65 ml	Four (4) vials, each containing mouse IgG with anti-E. coli HCP levels in arbitrary Activity Units; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
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	1% 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-Mouse Ig-HRP Concentrate.
- Graduated cylinder to dilute Wash Concentrate and Sample Diluent concentrate; 200ml to 1L.
- 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

Culture medium, 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. For other samples, including **tissue culture media**, clarify the sample by centrifugation and/or filtration prior to dilution in Working Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for long-term storage. Avoid freeze-thaw cycles.

Antibody Stability

Initial dilution of serum into **Working Sample Diluent** (WSD) 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** (LNSD), which provides the lowest assay background, should be at least 10 times the initial dilution and performed the same day as the assay.

Example: Initial (1/5): **10**ul serum + **40**ul WSD [or 0.1ml + 0.4ml]
Further (1/50): **10**ul initial (1/5) + **90**ul LNSD (1/50)

Assay Design

Review Calculation of Results (p5-7) and Limits of the Assay (above) 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 <0.4 OD. This is usually 1/100 or greater dilution for mouse sera with normal levels of IgG and IgM.
- 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 (**See Method A)**.
- Run a set of Calibrators. Calibrators validate that the assay was performed to specifications; results can be used to normalize between-assay variation for enhanced precision.
- Run a range of sample dilutions for expected higher positives that allows calculation of antibody **Titer** (when specific titer is at least 4-fold higher than non-immune)**.**

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-Mouse Ig-HRP Conjugate 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 Anti-Mouse Ig-HRP contain bromo-nitro-dioxane (BND: 0.05%, w/v). Stop Solution contains 1% 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, if not already on file, can be requested or obtained from the ADI website.