Mouse Anti-Type II Collagen IgG Assay Kit (Quantitative, mouse)

Item No. 500470
Materials Supplied

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item</th>
<th>Quantity/Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>400465</td>
<td>Mouse Type II Collagen Precoated EIA Strip Plate</td>
<td>1 plate</td>
</tr>
<tr>
<td>400407</td>
<td>Mouse Anti-Type II Collagen Polyclonal IgG Standard</td>
<td>1 vial</td>
</tr>
<tr>
<td>400071</td>
<td>Anti-Mouse IgG/HRP Conjugate</td>
<td>1 vial/1.2 ml</td>
</tr>
<tr>
<td>400060</td>
<td>EIA Buffer Concentrate (10X)</td>
<td>2 vials/10 ml</td>
</tr>
<tr>
<td>400062</td>
<td>Wash Buffer Concentrate (400X)</td>
<td>1 vial/5 ml</td>
</tr>
<tr>
<td>400035</td>
<td>Polysorbate 20</td>
<td>1 vial/3 ml</td>
</tr>
<tr>
<td>400074</td>
<td>TMB Substrate Solution</td>
<td>1 vial/12 ml</td>
</tr>
<tr>
<td>10011355</td>
<td>HRP Stop Solution</td>
<td>1 vial/12 ml</td>
</tr>
<tr>
<td>400012</td>
<td>96-Well Cover Sheet</td>
<td>3 covers</td>
</tr>
</tbody>
</table>

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.
WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.

Precautions

Please read these instructions carefully before beginning this assay.

The reagents in this kit have been tested and formulated to work exclusively with Cayman’s Mouse Anti-Type II Collagen IgG Assay Kit (mouse). This kit may not perform as described if any reagent or procedure is replaced or modified. The Stop Solution provided with this kit is an acid solution. Please wear appropriate personal protection equipment (e.g., safety glasses, gloves, and lab-coat) when using this material.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888
Fax: 734-971-3641
Email: techserv@caymanchem.com
Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored as directed at 4°C and used before the expiration date indicated on the outside of the box. Do not freeze this kit!

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm.
2. Adjustable pipettes and a repeat pipettor.
3. A source of ‘UltraPure’ water. Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants (‘UltraPure’). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. NOTE: UltraPure water is available for purchase from Cayman (Item No. 400000).
4. Materials used for Sample Preparation (see page 9).
INTRODUCTION

Background

Collagen-induced arthritis (CIA) is an experimental autoimmune disease that is widely used as a model of human rheumatoid arthritis (RA) in the study of the pathogenic mechanisms of this disease and for testing potential therapeutics. CIA is elicited in susceptible strains of rodents (mouse and rat) and nonhuman primates after immunization with heterologous type II collagen (CII), the major constituent protein of articular cartilage. Following immunization, these animals develop an autoimmune-mediated polyarthritis that shares several clinical, histological, immunological, and genetic features with human RA. The immune response to CII is characterized by both the stimulation of collagen-specific T cells and the production of high titers of antibody reactive to both heterologous CII and autologous CII, with the latter being most critical for the development of CIA. Antibody generated against type II collagen localizes in the joint of the host by binding to the intact autologous collagen. This event is followed by the activation of the complement and the initiation of the inflammation cascade, consisting of pro-inflammatory cytokines, chemokines, and cartilage degrading enzymes. Because circulating levels of type II collagen antibody are strongly associated with the development of arthritis, this antibody can be used as a biomarker of CIA.

About This Assay

Cayman’s Mouse Anti-Type II Collagen IgG Assay is an immunometric (i.e., sandwich) assay which can be used to measure type II collagen antibody in plasma or serum without prior sample purification. Cayman is offering this assay on three kinds of collagen coated plates: bovine, chick, and mouse. Two options are available: qualitative (bovine and chick) as well as quantitative (bovine, chick, and mouse).

In the qualitative kits, a monoclonal type II collagen antibody is used as a standard. This standard provides a highly reproducible qualitative estimate of the amount of polyclonal CII antibody in a mouse plasma or serum sample. But, because it is a monoclonal antibody, it does not completely reflect the full spectrum of polyclonal binding activity. In the quantitative kits, affinity-purified polyclonal antibody isolated from mice with CIA is used as a standard. While more expensive, this standard provides the most accurate measurement of anti-CII concentration in experimental plasma samples.

This kit uses a mouse collagen-coated plate and an anti-type II collagen polyclonal antibody standard. One unit of standard in this assay is approximately equal to 1 ng of IgG protein. The standard curve spans the range of 15.6-1,000 U/ml, with an LLOQ of 15.6 U/ml.

Description of Immunometric EIAs

Each well of the microwell plate supplied in the kit has been coated with mouse type II collagen. Autoantibodies reactive to type II collagen, if present in the biological fluid sample, will bind to the immobilized collagen. A detection antibody recognizing mouse IgG is added to the well. This antibody is labeled with HRP allowing quantification of the autoantibody. Addition of the HRP substrate 3,3',5,5'-tetramethylbenzidine (TMB), followed by Stop Solution produces a yellow colored product which can be measured spectrophotometrically. The intensity of the color is directly proportional to the amount of bound Anti-mouse IgG/HRP, which is proportional to the concentration of the type II collagen autoantibody.

$$\text{Absorbance} \propto [\text{Anti-mouse IgG/HRP}] \propto [\text{Collagen autoantibody}]$$

A schematic of this process is shown in Figure 1, on page 8.
NOTE: Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants (‘UltraPure’). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. UltraPure water may be purchased from Cayman (Item No. 400000).

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for about two months

1. EIA Buffer Preparation

Dilute the contents of each vial of EIA Buffer Concentrate (10X) (Item No.400060) with 90 ml of UltraPure water. Be certain to rinse the vial to remove any salts that may have precipitated. NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with water.

2. Wash Buffer Preparation

5 ml vial Wash Buffer Concentrate (400X) (Item No. 400062): Dilute to a total volume of 2 L with UltraPure water and add 1 ml of Polysorbate 20 (Item No. 400035). NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a regular pipette. A positive displacement pipette or a syringe should be used to deliver small quantities accurately.

Sample Preparation

Prior to use, it is recommended that mouse serum or plasma samples be diluted in EIA Buffer at least 1:5,000 in order to fall within the range of the standard curve. In general, mouse serum or plasma (prepared using heparin or EDTA as the anticoagulant) can be used directly in the assay following dilution in EIA Buffer.
Preparation of Assay-Specific Reagents

Mouse Anti-Type II Collagen Polyclonal IgG Standard

Reconstitute the contents of the Mouse Anti-Type II Collagen Polyclonal IgG Standard (Item No. 400407) with 1 ml of EIA Buffer. Mix gently. The concentration of this solution (the bulk standard) will be 1,000 U/ml. Label this solution #1.

To prepare the standard for use in the EIA: Obtain seven clean test tubes or microcentrifuge tubes and label them #2 through #8. Aliquot 500 µl of EIA Buffer into tubes #2-8. Serially dilute the standard by transferring 500 µl from the bulk standard to tube #2. Mix gently. Next, remove 500 µl from tube #2 and place into tube #3. Mix gently. Repeat this process for tubes #4-7. Do not add any antibody to tube #8. This tube contains no antibody, and serves as an indicator of non-specific binding (NSB). If desired, the value of this sample can be subtracted from all other standard and sample values. These diluted standards should be stored at 4°C and used within ten days.

Anti-Mouse IgG/HRP Conjugate

This reagent is supplied as a concentrated stock solution of donkey anti-mouse IgG polyclonal antibody conjugated to HRP. On the day of the assay, prepare a working solution by adding 0.43 ml of the Anti-Mouse IgG/HRP Conjugate (Item No. 400071) to 11.57 ml Assay Buffer (12 ml total). This working solution should be stable for 24 hours at 4°C, protected from light. In the event that two or more experiments are performed with this kit more than 24 hours apart, sufficient stock solution has been provided to produce additional 12 ml of the working solution.

Plate Set Up

The 96-well plate(s) included with this kit is supplied ready to use. It is not necessary to rinse the plate(s) prior to adding the reagents. NOTE: If you do not need to use all of the strips at once, place the unused strips back in the plate packet and store according to the plate insert at 4°C. Be sure the packet is sealed with the desiccant inside.

Each plate or set of strips must contain an eight point standard curve run in duplicate. NOTE: Each assay must contain this minimum configuration in order to ensure accurate and reproducible results. Each sample should be assayed at a minimum of two dilutions and each dilution should be assayed in duplicate. For statistical purposes, we recommend assaying samples in triplicate.

A suggested plate format is shown in Figure 3, on page 12. The user may vary the location and type of wells present as necessary for each particular experiment. The plate format provided below has been designed to allow for easy data analysis using a convenient spreadsheet offered by Cayman (see page 14, for more details). We suggest you record the contents of each well on the template sheet provided (see page 23).

Figure 2. Preparation of the Anti-Collagen standards
Figure 3. Sample plate format

Performing the Assay

Pipetting Hints

- Use different tips to pipette each reagent.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (i.e., slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.

Addition of Standards and Samples and First Incubation

1. Add 100 μl of the standards or diluted sample to the appropriate wells on the plate. Each sample should be assayed in duplicate, triplicate recommended.

2. Cover the plate with a 96-Well Cover Sheet (Item No. 400012). Incubate for two hours at room temperature on an orbital shaker.

Addition of Anti-Mouse IgG/HRP Conjugate and Second Incubation

1. Empty the wells and rinse four times with Wash Buffer. Each well should be completely filled with Wash Buffer during each wash. Invert the plate between wash steps to empty the fluid from the wells. After the last wash, gently tap the inverted plate on absorbent paper to remove the residual Wash Buffer.

2. Add 100 μl of the diluted Anti-Mouse IgG/HRP Conjugate to each well of the plate.

3. Cover the plate with a 96-Well Cover Sheet and incubate for one hour at room temperature on an orbital shaker.

Development of the Plate

1. Empty the wells and rinse four times with Wash Buffer.

2. Add 100 μl of TMB Substrate Solution (Item No. 400074) to each well of the plate.

3. Cover the plate with a 96-Well Cover Sheet and incubate for 10-20 minutes at room temperature in the dark. Development of the blue color can be monitored at 650 nm. When the maximum standard (S1) O.D. value reaches 0.5-0.6, Stop Solution should be added to the entire plate.

4. DO NOT WASH THE PLATE. Add 100 μl of HRP Stop Solution (Item No. 10011355) to each well of the plate. Blue wells will turn yellow and colorless wells will remain colorless. NOTE: The Stop Solution in this kit contains an acid. Wear appropriate protection and use caution when handling this solution.

Reading the Plate

1. Wipe the bottom of the plate with a clean tissue to remove fingerprints, dirt, etc.

2. Read the plate at a wavelength of 450 nm.
Many plate readers come with data reduction software that plots data automatically. Alternatively a spreadsheet program can be used. NOTE: Cayman has a computer spreadsheet available for data analysis. Please contact Technical Service or visit our website (www.caymanchem.com/analysis/immuno) to obtain a free copy of this convenient data analysis tool.

Calculations

Plotting the Standard Curve and Determining the Sample Concentration

Using computer reduction software, plot absorbance (linear y-axis) versus concentration (logarithmic x-axis) for standards (S1-S7) and fit the data with a 4-parameter logistic equation. Using the equation of the line, calculate the concentration of IgG in each sample. NOTE: The choice of x and y-axis (linear or logarithmic) is arbitrary and can be altered at the discretion of the user without changing the results of the assay.

Performance Characteristics

Sample Data

The standard curve presented here is an example of the data typically produced with this kit; however, your results will not be identical to these. You must run a new standard curve. Do not use the data below to determine the values of your samples. Your results could differ substantially. This assay typically develops in 10-20 minutes; i.e., the usual absorbance for the 1,000 U/ml standard is 1.0-3.0.

<table>
<thead>
<tr>
<th>Anti-Type II Collagen IgG (U/ml)</th>
<th>Absorbance (450 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000</td>
<td>2.739</td>
</tr>
<tr>
<td>500</td>
<td>1.781</td>
</tr>
<tr>
<td>250</td>
<td>1.073</td>
</tr>
<tr>
<td>125</td>
<td>0.613</td>
</tr>
<tr>
<td>62.5</td>
<td>0.344</td>
</tr>
<tr>
<td>31.3</td>
<td>0.183</td>
</tr>
<tr>
<td>15.6</td>
<td>0.131</td>
</tr>
<tr>
<td>0</td>
<td>0.049</td>
</tr>
</tbody>
</table>

Table 1. Typical results
Use data with confidence

Type II Collagen Antibody Standard curve
- Type II Collagen Antibody Intra-assay variation
- Type II Collagen Antibody Inter-assay variation

Figure 4. Typical standard curve

Precision:
The intra- and inter-assay CVs have been determined at multiple points on the standard curve. These data are summarized in the graph on page 16 and in the table below.

<table>
<thead>
<tr>
<th>Anti-Type II Collagen IgG (U/ml)</th>
<th>%CV* Intra-assay variation</th>
<th>%CV* Inter-assay variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000</td>
<td>6.22</td>
<td>2.59</td>
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<tr>
<td>500</td>
<td>4.09</td>
<td>2.16</td>
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<td>250</td>
<td>4.07</td>
<td>2.68</td>
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<tr>
<td>125</td>
<td>5.67</td>
<td>3.39</td>
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<tr>
<td>62.5</td>
<td>6.15</td>
<td>5.89</td>
</tr>
<tr>
<td>31.3</td>
<td>5.85</td>
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<tr>
<td>15.6</td>
<td>9.57</td>
<td>4.82</td>
</tr>
<tr>
<td>0</td>
<td>†</td>
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Table 2. Intra- and inter-assay variation
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve.
†Outside of the recommended usable range of the assay.
<table>
<thead>
<tr>
<th>Anti-Type II Collagen IgG (U/ml)</th>
<th>Mean O.D.</th>
<th>Standard Deviation (S.D.)</th>
<th>O.D. - (1.64 x S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000</td>
<td>1.81</td>
<td>0.08</td>
<td>1.67</td>
</tr>
<tr>
<td>500</td>
<td>1.19</td>
<td>0.08</td>
<td>1.07</td>
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<tr>
<td>250</td>
<td>0.74</td>
<td>0.04</td>
<td>0.67</td>
</tr>
<tr>
<td>125</td>
<td>0.45</td>
<td>0.03</td>
<td>0.40</td>
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<tr>
<td>62.5</td>
<td>0.28</td>
<td>0.02</td>
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<tr>
<td>31.3</td>
<td>0.17</td>
<td>0.01</td>
<td>0.15</td>
</tr>
<tr>
<td>15.6</td>
<td>0.12</td>
<td>0.01</td>
<td>0.10</td>
</tr>
<tr>
<td>0</td>
<td>0.05</td>
<td>0.00</td>
<td>0.06*</td>
</tr>
</tbody>
</table>

*O.D. + (1.64 x S.D.)

**Table 3. Determination of LLOQ**

The lower limit of quantitation (LLOQ) is defined as the lowest standard concentration in which O.D. - (1.64 x S.D.) is higher than the blank value of O.D. + (1.64 x S.D.). The LLOQ is 15.6 U/ml.

### RESOURCES

#### Troubleshooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erratic values; dispersion of duplicates</td>
<td>A. Trace organic contaminants in the water source</td>
<td>A. Replace activated carbon filter or change source of UltraPure water</td>
</tr>
<tr>
<td></td>
<td>B. Poor pipetting/technique</td>
<td></td>
</tr>
<tr>
<td>Poor development (low signal) of standard curve</td>
<td>A. Plate required more development time</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B. Standard was diluted incorrectly</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C. Standard is degraded</td>
<td></td>
</tr>
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</table>
References


Related Products

Mouse Anti-Type II Collagen IgG Assay Kit (Qualitative, bovine) - Item No. 500400
Mouse Anti-Type II Collagen IgG Assay Kit (Quantitative, bovine) - Item No. 500410
Mouse Anti-Type II Collagen IgG Assay Kit (Qualitative, chick) - Item No. 500420
Mouse Anti-Type II Collagen IgG Assay Kit (Quantitative, chick) - Item No. 500430
PAD4 Autoantibody EIA Kit - Item No. 500930
Warranty and Limitation of Remedy

Cayman Chemical Company makes no warranty or guarantee of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman warrants only to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have any obligation or liability, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer’s exclusive remedy and Cayman’s sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman’s option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.