Easy-Titer® Human IgM Assay Kit

23315  Description

23315  Easy-Titer Human IgM Assay Kit, contains sufficient components for 96 determinations

Kit Contents:

Anti-Human IgM Sensitized Beads, 2 ml, goat anti-human IgM coated polystyrene beads stabilized in a phosphate buffer, pH 7.4, containing bovine serum albumin and 0.1% sodium azide

Dilution Buffer, 30 ml, contains 0.1% sodium azide

Blocking Buffer, 15 ml, contains 0.1% sodium azide

Note: An IgM standard must be purchased separately.

Storage: Upon receipt store all kit components at 4°C. Kit is shipped at ambient temperature. Do not freeze kit components.

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Introduction

The Thermo Scientific Easy-Titer Human IgM Assay Kit is a simple, mix-and-read assay that allows quick and accurate determination of concentrations from 15-500 ng/ml of intact IgM. The assay procedure uses monodispersed polystyrene beads that are coated with anti-human IgM antibodies and absorb light at 340 and 405 nm. When the beads are mixed with a sample containing human IgM, they aggregate, causing decreased absorption of light and, therefore, low IgM concentrations yield high absorbance values and high IgM concentrations yield low absorbance values. The decrease in absorption is proportional to IgM concentration and a standard curve can be generated to accurately quantify IgM levels in serum or citrated plasma samples. The Easy-Titer Human IgM Assay Kit features a simple procedure with highly reproducible results.
**Procedure Summary**

1. Suspend Anti-Human IgM Sensitized Beads.
2. Prepare standards and sample(s).
3. Add 20 µl of the beads to the wells.
4. Add 20 µl of the sample to the wells.
5. Incubate on a plate mixer for 5 minutes at room temperature.
6. Add 100 µl of Blocking Buffer to the wells.
7. Mix plate for 5 minutes on a plate mixer.
8. Measure the absorbance at 405 nm or 340 nm. Plot standard curve to determine sample concentration.

**Important Product Information**

- Human bodily fluid must be handled and treated as a potentially infectious agent. Please adhere to local regulations for handling and disposal of infectious waste.
- Assay IgM standards and samples in triplicate. This kit produces acceptable assay precision when samples are assayed in triplicate. The coefficient of variation (CV) is typically ≤5% within an assay and ≤10% between assays.
- Absorbance values will often vary with each specific kit lot and with each assay performed. Always prepare a new human IgM standard curve for each assay.
- The Anti-Human IgM Sensitized Beads may settle in the cap area during shipping and storage. Mix the Anti-Human IgM Sensitized Beads end-over-end on a rotator for at least 10 minutes. Just before dispensing, vortex the beads vigorously for 60 seconds. The combination of end-over-end and vortex mixing will ensure that the beads are monodispersed.
- The detection range of this assay is 15-500 ng/ml of human IgM. If sample concentration is unknown, make serial dilutions and assay each dilution. Typically, human serum or plasma contains between 0.6 mg/ml and 2.5 mg/ml of IgM; hybridoma cell culture contains 1-30 µg/ml of IgM; mouse ascites contain 1-10 mg/ml of monoclonal antibody.
- Equilibrate all kit reagents to room temperature before use.

**Additional Materials Required**

- Human IgM (whole molecule) for generating a standard curve (e.g., Product No. 31146)
- Pipettors to accurately deliver 10-1,000 µl
- 96-well microplate(s) (e.g., Product No. 15031 or 15041)
- Microplate mixer (vigorous mixing capability is required)
- Microplate reader that can measure absorbance at 405 nm or 340 nm

**Easy-Titer Human IgM Assay Procedure**

A. **Sensitized Bead Preparation**
   Mix the Anti-Human IgM Sensitized Beads end-over-end on a rotator for at least 10 minutes. Just before dispensing, vortex the beads vigorously for 60 seconds.

B. **Sample Preparation**
   Dilute all samples to 15-500 ng/ml using the Dilution Buffer (See Important Product Information section).
   **Example:** To accurately dilute serum samples 1:4,000, use a two-step dilution process by first adding 990 µl of the Dilution Buffer to 10 µl of serum sample (1:100 dilution). Dilute further by adding 10 µl of the 1:100 diluted serum to 390 µl of Dilution Buffer (1:40 dilution) to yield the final 1:4,000 dilution.
C. Human IgM Standard Preparation

1. Use microcentrifuge tubes to prepare a 500 ng/ml working standard of the IgM. Often a large dilution is necessary to prepare the working standard and, therefore, perform a two step dilution to ensure accuracy.

   Example: Dilute the human IgM 1:100 using the Dilution Buffer, and then further dilute to a final concentration of 500 ng/ml. If the starting concentration of the human IgM is 2.4 mg/ml, then it needs to be diluted 1:4,800 (2.4 x10^6 ng/ml divided by 500 ng/ml = 4,800). Perform a 1:100 dilution, and then a 1:48 dilution (i.e., 20 µl of the 1:100 diluted IgM added to 940 µl of the Dilution Buffer).

2. Use a 96-well microplate or microcentrifuge tubes to make a 1:1 serial dilutions of the 500 ng/ml working standard for a total of seven standards and one blank. Only values obtained from standards 15.6 ng/ml to 500 ng/ml are used for calculations; however, the blank and 7.8 ng/ml standard are evaluated to verify kit performance.

   Example: Add 100 µl of the Dilution Buffer to wells B1 through H1. Add 100 µl of the 500 ng/ml working IgM standard to wells A1 and B1. Mix the contents of well B1 and transfer 100 µl to well C1. Mix the contents of well C1 and transfer 100 µl to well D1. Continue the serial dilutions for wells E1, F1 and G1. There is no transfer from well G1 to H1 because well H1 is the blank or zero point and contains only the Dilution Buffer.

D. Assay Protocol

- Perform all steps at room temperature.
- The timing of the incubation steps is not critical; however, for best results perform all incubations for 2-10 minutes.
- To avoid error, assay samples in triplicate.

1. Carefully pipette 20 µl of the Anti-Human IgM Sensitized Beads prepared in Section A into the appropriate number of wells in a 96-well microplate.

   Note: Work quickly to avoid settling of the sensitized beads. If dispensing the sensitized beads extends for longer than 60 seconds, recap the bottle and vortex beads for 5 seconds.

2. Carefully pipette 20 µl of the sample or standards (prepared in Sections B and C) into the appropriate wells containing the beads.

3. Mix the microplate continuously on a plate mixer at a moderate-to-high speed setting for 5 minutes.

   Note: Vigorous mixing is critical to ensure adequate and thorough integration of the sensitized beads with the sample.

4. Add 100 µl of the Blocking Buffer to each well.

5. To avoid spills, reduce the plate mixer speed to a moderate speed setting and mix continuously for 5 minutes.

6. Before evaluating the plate, remove or burst all large bubbles. Measure the absorbance at either 405 nm or 340 nm.

7. Generate a standard curve and determine the sample concentration from the standard curve. Include the dilution factor for each sample when determining its starting IgM concentration. Most calculations can be easily performed with the software available with most ELISA plate readers.

E. Calculations

The IgM concentration may be determined by interpolating between points on the curve. For example, a diluted sample yielded an absorbance of 0.715. Because the unknown has a value between the two standards 62.5 and 125 ng/ml (Table 1), the linear interpolation equation is as follows: 62.5 + 62.5[(0.854-0.715)/(0.854-0.704)] = 120 ng/ml. The serum sample was diluted 1:4,000 and, therefore, the IgM concentration in the original sample is 0.48 mg/ml.

Alternatively, the IgM concentration may be determined using the linear regression equation. For example, standard points were measured at 405 nm (Table 1) and standard concentrations were converted to the natural log to generate a curve (Figure 1). The diluted sample yielded an absorbance value of 0.715. Solving the linear regression equation (i.e., Y = -0.1863Ln(X) + 1.6108) for Ln(X) and substituting 0.715 for Y, yields a natural log value of 4.803. Converting the natural log to a real number reveals the IgM concentration to be 121.9 ng/ml. The serum sample was diluted 1:4,000 and, therefore, the IgM concentration in the original sample is 0.488 mg/ml.

Note: A nonlinear regression analysis also may be performed on the entire curve. Please refer to the computer software manual for information concerning nonlinear regression.
**Table 1.** Values obtained from the standard curve points measured at 405 nm.

<table>
<thead>
<tr>
<th>IgM Standard (ng/ml)</th>
<th>Ln IgM Standard (ng/ml)</th>
<th>Absorbance (405 nm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>500</td>
<td>6.215</td>
<td>0.457</td>
</tr>
<tr>
<td>250</td>
<td>5.521</td>
<td>0.568</td>
</tr>
<tr>
<td>125</td>
<td>4.828</td>
<td>0.704</td>
</tr>
<tr>
<td>62.5</td>
<td>4.135</td>
<td>0.854</td>
</tr>
<tr>
<td>31.2</td>
<td>3.440</td>
<td>1.002</td>
</tr>
<tr>
<td>15.6</td>
<td>2.747</td>
<td>1.071</td>
</tr>
</tbody>
</table>

*Values are averages of three wells.

**Figure 1.** Standard curve for Human IgM (suggested standard curve range: 15.6 ng/ml to 500 ng/ml). The data from Table 1 were used to plot the semi-log graph.

**Troubleshooting**

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poor precision (high CVs) for all standards and samples</td>
<td>Inadequate mixing of diluted sample with the beads</td>
<td>Mix the plate vigorously for 5 minutes</td>
</tr>
<tr>
<td>Poor precision for one sample or standard</td>
<td>Some of the wells contain bubbles</td>
<td>Burst bubbles with a gentle stream of air or with the tip of a pasteur pipette and measure the plate again</td>
</tr>
<tr>
<td>Low absorbance for the blank</td>
<td>Inadequate mixing of the beads</td>
<td>Mix the beads end-over-end for 10 minutes then vortex 10 seconds just before dispensing</td>
</tr>
<tr>
<td>( R^2 ) is less than 95%</td>
<td>Poor correlation between IgM standard curve and the linear regression line</td>
<td>Remake the serial dilutions of the IgM standard and repeat the assay</td>
</tr>
</tbody>
</table>

**Related Thermo Scientific Products**

- 31146 Human IgM, Whole Molecule, 2 mg
- 44887 IgM Fragmentation Kit
- 44897 IgM Purification Kit
- 53000 Pierce Fluorescein Protein Labeling Kit
- 23310 Easy-Titer Human IgG Assay Kit
- 23305 Easy-Titer Rabbit IgG Assay Kit
- 23300 Easy-Titer® Mouse IgG Assay Kit
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