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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

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MHC Class I (human) ELISA Kit

Item No. 502060

www.caymanchem.com
Customer Service 800.364.9897
Technical Support 888.526.5351
1180 E. Ellsworth Rd · Ann Arbor, MI · USA

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GENERAL INFORMATION

Materials Supplied

Item Number	Item Name	96 wells Quantity/Size	Storage Temperature
502061	MHC Class I (human) Standard	1 vial	-20°C
502062	Anti-Beta 2-Microglobulin HRP Conjugate	1 vial/1.5 ml	-20°C
502063	Anti-MHC Class I (Clone W6/32) ELISA Strip Plate	1 plate	-20°C
502064	Immunoassay Buffer E Concentrate (10X)	1 vial/10 ml	4°C
400062	Wash Buffer Concentrate (400X)	1 vial/5 ml	RT
400035	Polysorbate 20	1 vial/3 ml	RT
400074	TMB Substrate Solution	1 vial/12 ml	4°C
10011355	HRP Stop Solution	1 vial/12 ml	RT
400012	96-Well Cover Sheet	1 ea	RT

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 971-3335. We cannot accept any returns without prior authorization.

WARNING: THIS PRODUCT IS FOR RESEARCH ONLY - NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.

Safety Data

This material should be considered hazardous until further information becomes available. Do not ingest, inhale, get in eyes, on skin, or on clothing. Wash thoroughly after handling. Before use, the user must review the complete Safety Data Sheet, which has been sent *via* email to your institution.

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 Chemical's MHC Class I (human) ELISA Kit. 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.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Email: techserv@caymanchem.com

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 in the **Materials Supplied** section (see page 3) and used before the expiration date indicated on the outside of the box.

Materials Needed But Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm
2. An orbital microplate shaker
3. Adjustable pipettes; multichannel or repeating pipettor recommended
4. A source of ultrapure water, with a resistivity of 18.2 MΩ·cm and total organic carbon (TOC) levels of <10 ppb, is recommended. Pure water - glass-distilled or deionized - may not be acceptable. *NOTE: UltraPure Water is available for purchase from Cayman (Item No. 400000).*
5. Materials used for **Sample Preparation** (see page 10)

INTRODUCTION

Background

Major histocompatibility complex (MHC) molecules, also designated human leukocyte antigen (HLA) molecules in humans, are glycoproteins that aid in T cell mediated immunity through the presentation of pre-loaded antigenic peptides.[1,2] MHC class I molecules consist of an α or heavy chain, which contains the variable region, non-covalently linked to the β chain, beta 2-microglobulin.[3-5] They are assembled in the endoplasmic reticulum of all nucleated cells. Following assembly and intracellular peptide association, MHC class I molecules exit the endoplasmic reticulum through a complex secretory pathway and present the peptide antigen at the cell surface to cytotoxic $CD8^+$ T cells, initiating antitumor or antiviral responses.[2,6] MHC class I molecules can also present extracellular peptide antigens through a process known as cross-presentation.[7] Various altered MHC class I phenotypes, including haplotypic loss, allelic loss, and total MHC class I loss, have been found in human cancer cells and are associated with immune evasion.[6] Pharmacologic reduction of MHC class I expression reduces incidence and disease severity in animal models of experimental and spontaneous autoimmune systemic lupus erythematosus (SLE).[8] The MHC Class I (human) ELISA Kit recognizes peptide-bound and fully-assembled MHC class I molecules.

About This Assay

Cayman's MHC Class I (human) ELISA Kit is an immunometric assay (*i.e.* sandwich) that can be used for the quantification of MHC Class I in human cell lysates, cell supernatants, serum, and plasma. The standard curve spans the range of 1.6-100 ng/ml, with a lower limit of quantification (LLOQ) of 1.6 ng/ml.

Principle Of This Assay

This immunometric assay is based on a double-antibody "sandwich" technique. Each well of the microwell plate supplied with the kit has been coated with a monoclonal antibody specific for human MHC Class I. This antibody will bind any human MHC Class I introduced into the well. A second monoclonal antibody conjugated to horseradish peroxidase (HRP), which recognizes Beta 2-Microglobulin, is added to the well forming a "sandwich". The "sandwich" is immobilized on the plate and the excess reagents are washed away. The concentration of the human MHC Class I is determined by measuring the enzymatic activity of HRP using the chromogenic substrate TMB. After a sufficient period, the reaction is stopped with acid, forming a product with a distinct yellow color that can be measured at 450 nm. The intensity of the color is directly proportional to the amount of bound antibody-HRP conjugate, which is proportional to the concentration of human MHC Class I.

Absorbance $\propto$ [Anti-Beta 2-Microglobulin-HRP] $\propto$ [MHC Class I (human)]

A schematic of this process is shown in Figure 1, on page 8.

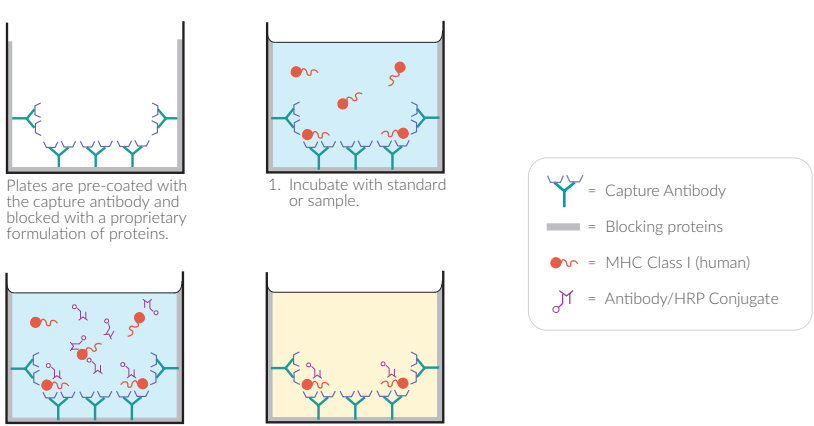


Figure 1. Schematic of the ELISA

Definition of Key Terms

Standard Curve: a plot of the absorbance values *versus* concentration of a series of wells containing various known amounts of analyte.

Lower Limit of Detection (LLOD): the smallest measure that can be detected with reasonable certainty for a given analytical procedure. The LLOD is defined as a concentration two standard deviations higher than the mean zero value.

Lower Limit of Quantification (LLOQ): the lowest standard concentration in which absorbance (450 nm) – (1.64 x S.D.) is higher than the mean zero value of absorbance (450 nm) + (1.64 x S.D.).

PRE-ASSAY PREPARATION

Buffer Preparation

Store all diluted buffers at 4°C; they will be stable for approximately two months. NOTE: It is normal for the concentrated buffer to contain crystalline salts after thawing. These will completely dissolve upon dilution with ultrapure water.

1. **Immunoassay Buffer E (1X) Preparation**

   Dilute the contents of one vial of Immunoassay Buffer E Concentrate (10X) (Item No. 502064) with 90 ml of ultrapure water. Be certain to rinse the vial to remove any salts that may have precipitated.

2. **Wash Buffer (1X) Preparation**

   Dilute the contents of one vial of Wash Buffer Concentrate (400X) (Item No. 400062) with ultrapure water to a total volume of 2 L and add 1 ml of Polysorbate 20 (Item No. 400035). Smaller volumes of Wash Buffer (1X) can be prepared by diluting the Wash Buffer Concentrate (400X) 1:400 and adding 0.5 ml of Polysorbate 20 per 1 L of Wash Buffer (1X). *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

Sample Collection and Storage

This assay has been validated in human plasma and serum, as well as human cell lysates and supernatants, without purification. Other sample types, lysis buffers not described in this section, or concentrated cell lysates may cause interference and require a minimum dilution determined by the end user. Please read this section thoroughly before beginning the assay.

Testing for Interference

To test for interference, dilute one or two test samples to obtain several different dilutions for each sample. The dilution factor where the change in the final calculated human MHC Class I concentration is consistent, differing by 20% or less than the previous dilution, is the minimum required dilution for that particular sample type.

Plasma

Collect blood in vacutainers containing EDTA or Heparin. To obtain plasma, centrifuge blood at 1,000 x g for 15 minutes. Pipette off the top plasma layer without disturbing the white buffy layer. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.

Serum

Collect blood in vacutainers without a coagulant for serum samples. Allow samples to clot undisturbed for 30-60 minutes at room temperature. To obtain serum, centrifuge at 1,000-2,000 x g for 15-30 minutes. Pipette off the serum layer. Samples should be assayed immediately after collection; samples that cannot be assayed immediately should be stored at -80°C.

Cell Lysates

This assay has been demonstrated to work with human cells (see Figure 3 on page 13) lysed in Pierce™ IP Lysis Buffer (available from Thermo Fisher Scientific) with added protease inhibitors (cOmplete™, Mini, EDTA-free Protease Inhibitor Cocktail available from MilliporeSigma). It is recommended to lyse cells on ice for 30 minutes before spinning at 20,000 x g at 4°C for 30 minutes. Collect supernatant and assay immediately or aliquot and store at -20°C.

Cell Supernatants

All cell supernatants MUST be diluted at least 1:2 with Immunoassay Buffer E (1X) prior to use in this assay. A minimum volume of 200 µl of each diluted sample is needed to run the samples in duplicate in the assay; for convenience, we recommend preparing 250 µl of each diluted sample.

Sample Matrix Properties

Parallelism

To assess parallelism, various matrices were assayed at multiple dilutions using the MHC Class I (human) ELISA Kit. Concentrations were plotted as a function of sample dilution. The results are shown below.

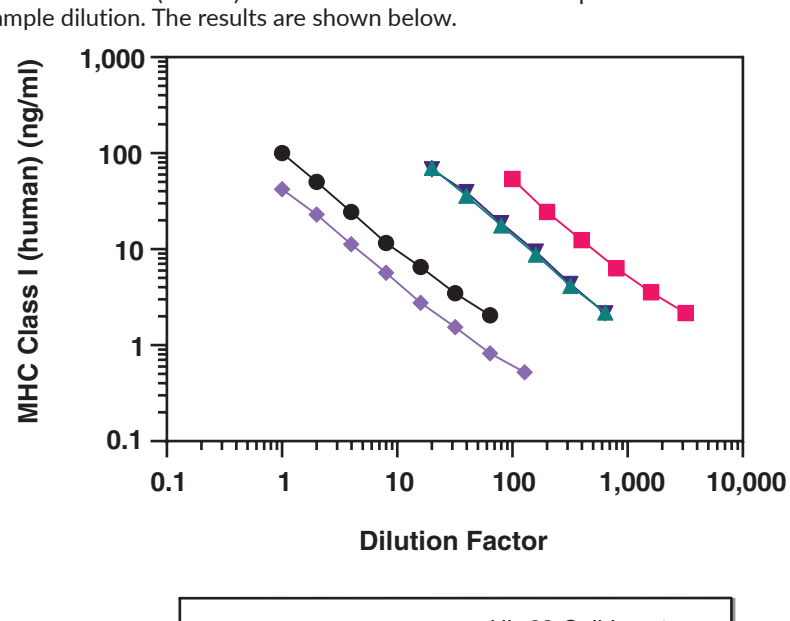


Figure 2. Parallelism of various matrices in the MHC Class I (human) ELISA Kit

Cell Line Validation

The indicated cell lines were lysed at 10 million cells per ml as described in the Sample Preparation section. Daudi cells (Beta 2-microglobulin negative) and RAW 264.7 murine macrophages were included as negative controls. The error bars represent standard deviations obtained from multiple dilutions of each sample.

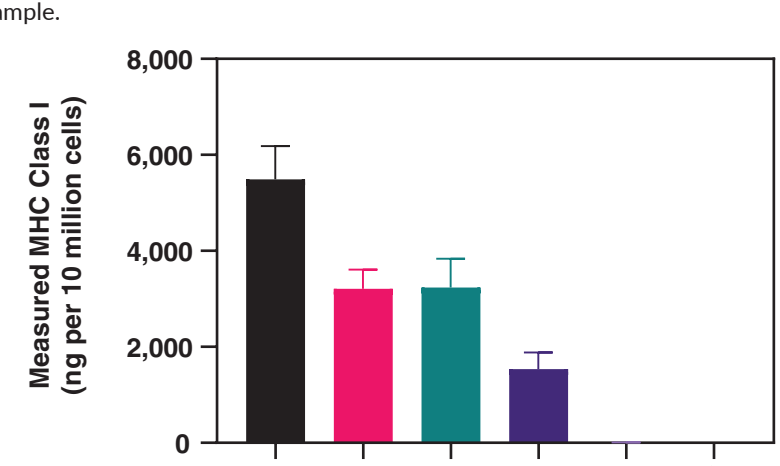


Figure 3. Native MHC Class I (human) levels in various cell lines

Spike and Recovery

For validation in cell supernatant, cell culture media (RPMI-1640 + 10% fetal bovine serum) was spiked with different amounts of MHC Class I. For validation in cell lysates, Daudi cells (Beta 2-microglobulin negative) were lysed at 10 million cells per ml as described in the Sample Preparation section, spiked with different amounts of HL-60 (MHC Class I high) cell lysates prepared in the same manner. All samples were diluted with Immunoassay Buffer E (1X) and analyzed using the MHC Class I (human) ELISA Kit. The error bars represent standard deviations obtained from multiple dilutions of each sample.

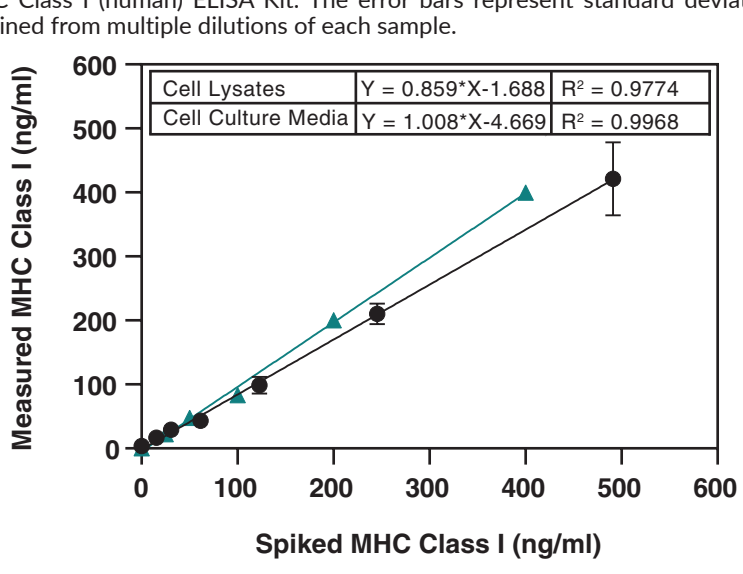


Figure 4. Spike and recovery in cell culture media and cell lysates

Human plasma and serum were mixed at the ratios indicated below, diluted, and analyzed using the MHC Class I (human) ELISA Kit. The results are shown in the tables below. The expected concentration is based off the ratios used.

High Serum	Low Serum	Expected Concentration (ng/ml)	Measured Concentration (ng/ml)	% Recovery
0%	100%	--	1,425	--
25%	75%	3,831	3,306	86.3
50%	50%	6,236	6,015	96.5
75%	25%	8,642	6,877	79.6
100%	0%	--	11,047	--
High Plasma	**Low Plasma**	**Expected Concentration (ng/ml)**	**Measured Concentration (ng/ml)**	**% Recovery**
0%	100%	--	1,736	--
25%	75%	3,495	3,844	110.0
50%	50%	5,255	5,665	107.8
75%	25%	7,014	7,182	102.4
100%	0%	--	8,773	--

Table 1. Spike and recovery of MHC Class I (human) in various matrices

Linearity

HL-60 cell lysates (cells were lysed at 10 million cells per ml as described in the Sample Preparation section), Jurkat cell supernatant, human plasma, and serum were assay at multiple dilutions using the MHC Class I (human) ELISA Kit. The results are shown the Table 2, on page 17.

NOTE: Linearity has been calculated using the following formula:
*%Linearity = (Observed concentration value, dilution adjusted/First observed concentration value in the dilution series, dilution adjusted)*100*

Dilution	Concentration (ng/ml)	Dilutional Linearity (%)
HL-60 Cell Lysates		
200	17,430	100.0
400	17,993	103.2
800	18,815	107.9
1,600	18,077	103.7
Jurkat Cell Supernatant		
2	45.93	100.0
4	45.19	98.4
8	45.42	98.9
16	44.39	96.7
Human Plasma		
20	1,412	100.0
40	1,444	102.3
80	1,416	100.3
160	1,408	99.7
Human Serum		
20	1,360	100.0
40	1,571	115.5
80	1,493	109.8
160	1,511	111.1

Table 2. Dilutional linearity of various matrices

Preparation of Assay-Specific Reagents

MHC Class I (human) Standard

Reconstitute the lyophilized MHC Class I (human) Standard (Item No. 502061) with 0.5 ml of Immunoassay Buffer E (1X) and mix gently. The concentration of this solution (the bulk standard) will be 1000 ng/ml. The reconstituted standard will be stable for approximately three weeks when stored at 4°C.

To prepare the standard for use in ELISA: Obtain eight clean test tubes and label them #1-8. Aliquot 900 µl Immunoassay Buffer E (1X) to tube #1 and 500 µl Immunoassay Buffer E (1X) to tubes #2-8. Transfer 100 µl of the bulk standard (1,000 ng/ml) to tube #1 and mix gently. Serially dilute the standard by removing 500 µl from tube #1 and placing it in tube #2; mix thoroughly. Next, remove 500 µl from tube #2 and place it into tube #3; mix thoroughly. Repeat this process for tubes #4-7. Do not add any MHC Class I (human) Standard to tube #8. This tube is the background control. These diluted standards should be used within three hours.

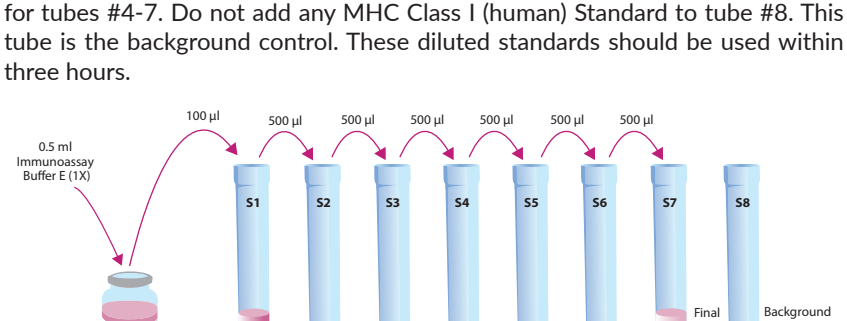


Figure 5. Preparation of the MHC Class I (human) standards

Anti-Beta 2-Microglobulin-HRP Conjugate

Anti-Beta 2-Microglobulin-HRP Conjugate (Item No. 502062) is supplied as a concentrated (10X) stock solution of Anti-Beta 2-Microglobulin antibody conjugated to HRP. On the day of the assay, thaw the antibody-HRP conjugate at room temperature.

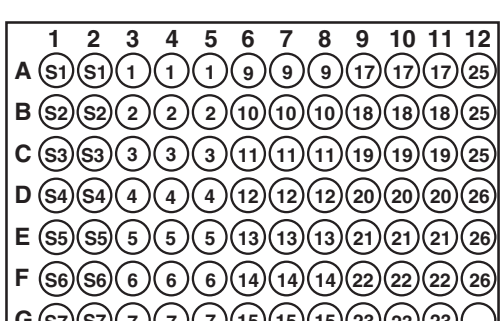
For a full plate, dilute 1.2 ml of the antibody-HRP conjugate into 10.8 ml of Immunoassay Buffer E (1X); for a half plate, dilute 0.6 ml of the antibody-HRP conjugate into 5.4 ml of Immunoassay Buffer E (1X) to make a 1X working solution. Do not prepare diluted antibody-HRP conjugate until immediately before use. Discard any unused antibody-HRP conjugate (1X). Store Anti-Beta 2-Microglobulin HRP Conjugate (10X) stock solution at 4°C.

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 the strips at once, place the unused strips back in the plate packet and store at -20°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 6, on page 20. The user may vary the location and type of wells present as necessary for each particular experiment. We suggest recording the contents of each well on the template sheet provided (see page 29).



S1-S8 - Standard Wells
1-26 - Sample Wells

Figure 6. 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. Pipette 100 µl of the MHC Class I (human) Standards or samples into the appropriate wells on the plate. Each sample should be assayed in duplicate, triplicate recommended.
2. Cover the plate with the 96-Well Cover Sheet (Item No. 400012), tap gently to mix, and incubate for two hours at room temperature on an orbital shaker.

Addition of the Anti-Beta 2-Microglobulin-HRP Conjugate and Second Incubation

1. Empty the wells and rinse five times with ~300 µl Wash Buffer (1X). After the last wash, gently tap the inverted plate on absorbent paper to remove the residual wash buffer.
2. Prepare a 1X working solution of the Anti-Beta 2-Microglobulin-HRP Conjugate as described in the Preparation of Assay-Specific Reagents section.
3. Add 100 µl of the Anti-Beta 2-Microglobulin-HRP Conjugate (1X) working solution to each well of the plate.
4. Cover the plate with the 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 five times with ~300 µl Wash Buffer (1X). After the last wash, gently tap the inverted plate on absorbent paper to remove the residual wash buffer.
2. Add 100 µl of TMB Substrate Solution (Item No. 400074) to each well of the plate.
3. Cover the plate with plastic film. Optimum development is obtained by using an orbital shaker at room temperature for 30 minutes, protected from light.
4. DO NOT WASH THE PLATE. Add 100 µl of HRP Stop Solution (Item No. 10011355) to each well of the plate. Blue wells should turn yellow and colorless wells should 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.

ANALYSIS

Many plate readers come with data reduction software that plot data automatically. Alternatively, a spreadsheet program can be used.

Calculations

Plot the Standard Curve and Determine the Sample Concentration

Using computer reduction software, plot absorbance (linear y-axis) *versus* concentration (linear x-axis) for standards (S1-S8) and fit the data with a linear fit. Using the equation of the line, calculate the concentration of MHC Class I (human) in each sample, making sure to correct for any sample dilution.

Performance Characteristics

Representative 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.

MHC Class I Standards (ng/ml)	Absorbance	%CV* Intra-assay Precision	%CV* Inter-assay Precision
100	2.323	5.4	4.8
50	1.171	4.2	5.0
25	0.558	3.4	7.8
12.5	0.298	4.2	7.6
6.3	0.172	5.1	7.8
3.1	0.110	4.3	8.7
1.6	0.086	3.2	10.9
0	0.046	--	--

Table 3. Typical results
*%CV represents the variation in concentration (not absorbance) as determined using a reference standard curve

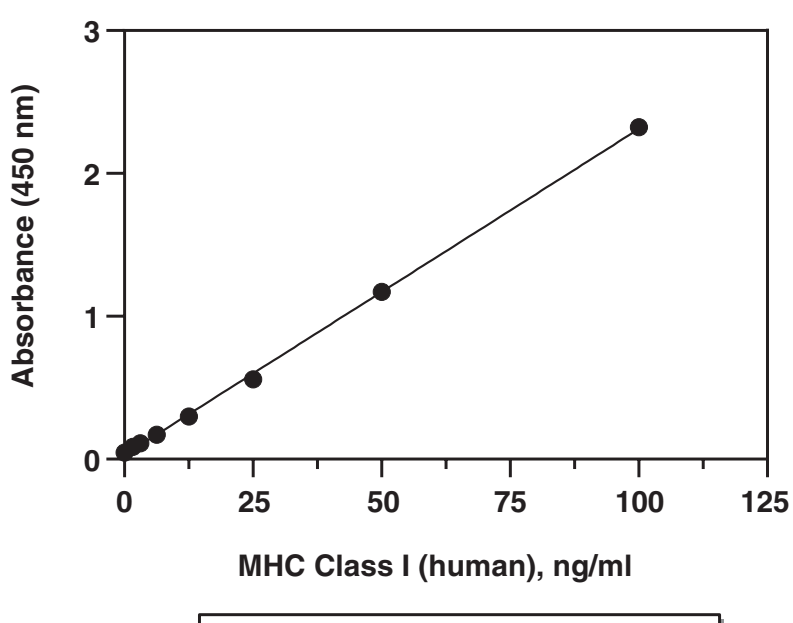


Figure 7. Typical standard curve

Precision:

Intra-assay precision was determined by analyzing 24 replicates of two human plasma controls in a single assay.

Matrix Control (ng/ml)	%CV
10,183	6.1
1,647	3.4

Table 4. Intra-assay precision

Inter-assay precision was determined by analyzing replicates of two human plasma controls in eight separate assays on different days.

Matrix Control (ng/ml)	%CV
12,029	8.3
1,954	10.5

Table 5. Inter-assay precision

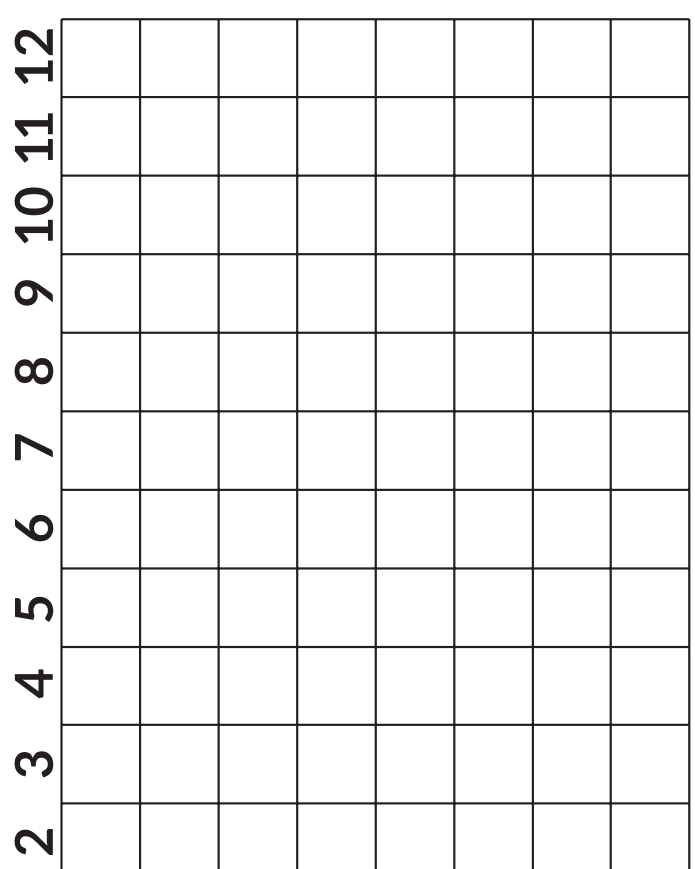
RESOURCES

Troubleshooting

Problem	Possible Causes
Erratic values; dispersion of duplicates	A. Trace organic contaminants in the water source B. Poor pipetting/technique
High background wells (>0.150 O.D.)	A. Poor washing; ensure proper washing is used B. Exposure of background wells to standards or samples
Poor development (low signal) of standard curve	A. Trace organic contaminants in the water source B. Dilution error in preparing reagents
Poor development (low signal) of samples	A. Insufficient lysis of cells; normalize to protein content of cell lysate B. Specific sample type is not MHC Class I (human) positive C. Samples are too dilute
Analyses of two dilutions of a biological sample do not agree (*i.e.*, more than 20% difference)	Interfering substances are present; determine minimal dilution for that sample type

Procedure	Standards/Samples
Mix all reagents gently	--
Add standards/samples to plate	100 µl
Seal	Seal the plate and tap gently to mix
Incubate	Incubate plate for 2 hours at room temperature on an orbital shaker
Aspirate	Aspirate wells and wash 5 x ~300 µl at room temperature
Apply antibody-HRP conjugate solution (1X)	100 µl
Incubate	Incubate plate for 1 hour at room temperature on an orbital shaker
Aspirate	Aspirate wells and wash 5 x ~300 µl with Wash Buffer (1X)
Apply TMB Substrate Solution	100 µl
Seal	Seal plate and incubate for 30 minutes at room temperature on an orbital shaker, protected from light
Apply HRP Stop Solution, do not wash	100 µl
Read	Read absorbance at 450 nm

Table 6. Assay Summary



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NOTES

Warranty and Limitation of Remedy

Buyer agrees to purchase the material subject to Cayman's Terms and Conditions. Complete Terms and Conditions including Warranty and Limitation of Liability information can be found on our website.