BioLegend®

Enabling Legendary Discovery™

LEGEND MAX™

ELISA Kit

Flex-T™ Human Class I Peptide Exchange ELISA

Cat. No. 447207

ELISA Kit for evaluation of UV activated peptide exchange for Flex-T™ Human MHC class I monomer

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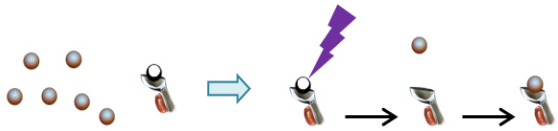
biolegend.com

It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

Table of Contents

Introduction:

BioLegend's Flex-T™ MHC products allow high flexibility in studying antigen specific T cells. Biotinylated MHC monomers are manufactured with a UV-labile peptide that can be degraded with an ultraviolet light source. This allows for a peptide exchange when UV irradiation is performed in the presence of a peptide of interest (shown in below graph). This flexibility permits the screening of virtually any peptide of interest with enough affinity for the MHC allele that it is loaded onto. Refer to the BioLegend Flex-T website for more details (https://www.biolegend.com/en-us/flex-t).



Peptide of interest mixed with labile peptide-loaded Flex-T™ monmers

UV light degrades labile peptide, allowing for substitution by peptide of interest

BioLegend's LEGEND MAX™ Flex-T™ HLA Class I ELISA kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a mouse monoclonal anti-human β2-microglobulin antibody. HRP-conjugated streptavidin is the detection reagent that binds to biotinylated HLA class I monomer. Through binding of β2-microglobulin subunit and biotin conjugated on the HLA class I monomer, this Sandwich assay detects the HLA class I α-chain/β2-microglobulin/peptide complex and only intact complex is recognized. Therefore, exchange product with high affinity peptides will show relatively high signals in this assay, while moderate or low affinity peptides exchange would result in relatively lower or non-detectable signals. A Flex-TTM HLA Class I ELISA Control is included in the kit as the assay control. This kit is not intended to be used to measure the actual affinity of peptides, but is specifically designed to qualitatively evaluate the efficiency of UV peptide exchange when loading peptides of interest into Flex-T™ HLA class I monomers. Each assay kit comes with analytically validated, ready-to-use reagents

Tel: 858-768-5800

Materials Provided:

Description	Quantity	Volume	Part #
Flex-T™ Pre-coated 96-well Strip Microplate	1 plate		750000621
Flex-T™ HLA Class I ELISA Control	1 vial	lyophilized	750000624
Avidin HRP	1 bottle	12 mL	77897
Assay Buffer A	2 bottles	25 mL each	78232
Wash Buffer (20x)	1 bottle	50 mL	78233
Substrate Solution F	1 bottle	12 mL	79132
Stop Solution	1 bottle	12 mL	79133
Plate Sealers	4 sheets		78101

Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Polypropylene vials to prepare control dilutions
- Timer
- Plate Shaker

Storage Information:

Store unopened kit components between 2°C and 8°C. Do not use this kit beyond its expiration date.

Opened or Reconstituted Components	
Microplate wells	If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store between 2°C and 8°C for up to one month.
Control	The remaining reconstituted control stock solution can be aliquoted into polypropylene vials and stored at 2~8 degrees for up to 48 hours. Avoid freeze-thaw cycles.
Avidin-HRP	Store opened reagents between 2°C and 8°C and use within one month.
Assay Buffer A	
Wash Buffer (20X)	
Substrate Solution F	
Stop Solution	

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details (www.biolegend.com/msds).
2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.
3. Stop Solution contains strong acid. *Wear eye, hand, and face protection.*
4. Before disposing of the plate, rinse it with an excess amount of tap water.

Specimen Collection and Handling:

The samples are UV-activated peptide exchange reaction mixture. It is recommended that samples be assayed immediately after peptide exchange. If not assayed immediately, samples can be stored at 2-8 degrees for up to 48 hours in tightly sealed plates or tubes and protected from light. Freeze thaws are not adviced for samples.

Reagent and Sample Preparation:

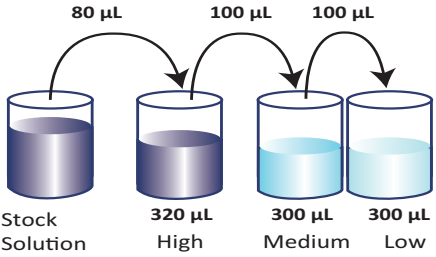
Note: All reagents should be diluted immediately prior to use.

1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the lyophilized Flex-T™ HLA Class I ELISA Control by adding the volume of Assay Buffer A to make the 50 ng/mL control stock solution (Refer to LEGEND MAX Kit Lot-Specific Certificate of Analysis/LEGEND MAX Kit Protocol). Allow the reconstituted control to sit at room temperature for 15-20 minutes, then briefly vortex to mix completely.
3. In general a 5 ng/mL concentration is adviced for all samples. For example, if the sample concentration is 25 ug/mL after exchange, a two-step dilution in Assay Buffer A (total 1:5000) is recommended for samples. First, prepare a 1:100 dilution of sample with 2 µL of sample in 198 µL of Assay Buffer A, then prepare a 1:50 dilution of the 1:100 diluted sample (e.g., 4 µL 1:100 diluted sample in 196 µL of Assay Buffer A). If further dilution is desired, dilution should be continually done with Assay Buffer A.

Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all samples be run in duplicate or triplicate.
2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.
3. Prepare 400 µL of the High control by adding 80 uL of the control stock solution in 320 µL Assay Buffer A. Perform 2 four-fold serial dilutions of the High control in separate tubes using Assay Buffer A as the diluent. Thus, the Flex-T™ HLA Class I ELISA Control with be prepapred in the High, Medium, and Low concentrations. Assay Buffer A serves as the zero control.



4. Add 50 µL of Assay Buffer A to each well that will contain either controls or samples. Then add 50 µL of control dilutions or samples to the appropriate wells.

5. Seal the plate with a Plate Sealer included in the kit and incubate the plate for 30 minutes at room temperature with shaking. If a Plate Shaker is not available, the sealed plate can be placed on bench at room temperature for 2 hours without shaking.

6. Discard the contents of the plate into a sink, wash the plate 4 times with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absobent paper.

7. Add 100 µL of Avidin-HRP solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking. If a Plate Shaker is not available, the sealed plate can be placed on bench at room temperature for 30 minutes without shaking.

8. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as described in step 6. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.

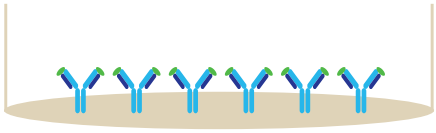
9. Add 100 µL of Substrate Solution F to each well and incubate for 10 minutes in the dark. Wells containing Flex-T™ HLA Class I should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.

10. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution color should change from blue to yellow.

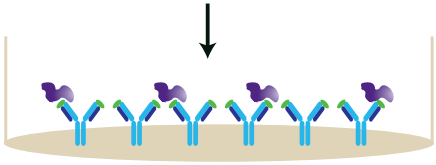
11. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

Assay Procedure Summary

1. Add 50 µL Assay Buffer A to control wells and sample wells

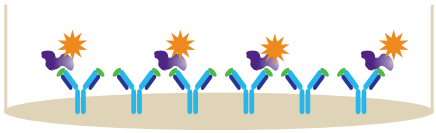


2. Add 50 µL of control or sample, incubate 30 min, RT, shaking



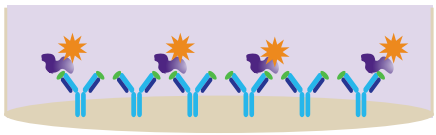
Wash 4 times

3. Add 100 µL Avidin-HRP solution Incubate 30 min, RT, shaking

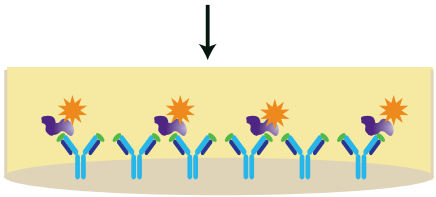


Wash 5 times

4. Add 100 µL Substrate Solution F Incubate 10 min, RT, in the dark



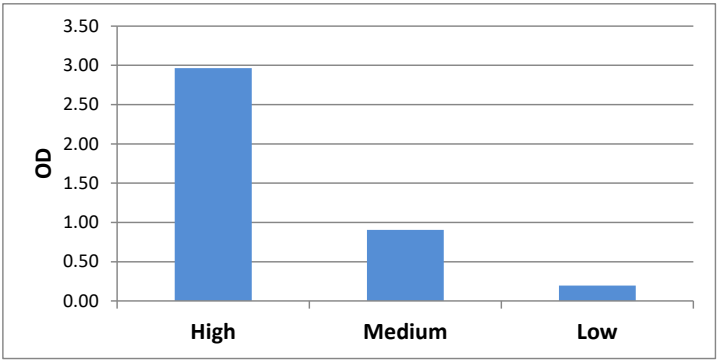
5. Add 100 µL Stop Solution



6. Read absorbance at 450 nm and 570 nm

Typical Data:

This High, Medium, and Low control graph was generated at BioLegend for demonstration purposes only. The optical density (OD) is calculated by absorbance at 450 subtracted with absorbance at 570 throughout this manual.



Performance Characteristics:

Specificity: This kit recognizes Flex-T™ Human Class I α-chain/β2-microglobulin/peptide complex.

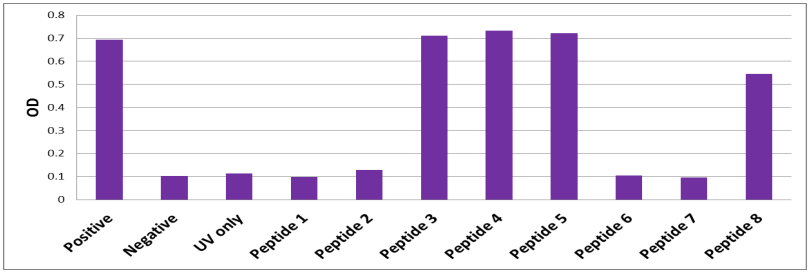
Linearity: Samples (n= 24) were first diluted to 5,000-fold with Assay Buffer A then serially diluted 1:2, 1:4, 1:8 with Assay Buffer A and assayed with the LEGEND MAX™ Flex-T™ Human HLA Class I ELISA kit. The measured ODs of serially diluted samples were then compared with the OD of the lowest dilution based on serial dilution used. The linearity calculated for this assay is 104%.

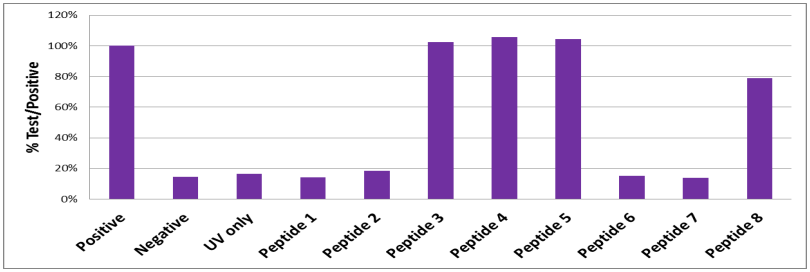
Intra-Assay Precision: Two samples containing different concentrations of Flex-T™ HLA Class I control were tested on one plate with 16 replicates (n=16). The intra- assay %CV of two samples are shown in below table.

Inter-Assay Precision: Two samples containing different concentrations of Flex-T™ HLA Class I control were tested in ten independent assays (n=10). The inter- assay %CV of two samples are shown in below table.

	Number of Assays	Sample 1	Sample 2
Intra-Assay %CV	16	11.2%	11.6%
Inter-Assay %CV	10	9.80%	13.6%

Samples: Flex-T™ HLA-A*02:01 UVX monomer (Cat# 280003) was treated with a 366nm UV in the presence of a Positive or a Negative control peptide, or test peptides (n=8), or no peptide (UV-only). After peptide exchange, each reaction mixture was then diluted following the instructions in "Reagent and Sample Preparation" section and analyzed by the ELISA. The ODs of each sample are shown in upper bar graph and the percentile (%) of test peptide against Positive control peptide in lower bar graph. Please refer to the BioLegend Flex-T website for details regarding data interpretation on UV-activated peptide exchange (https://www.biolegend.com/en-us/flex-t).





Troubleshooting Guide:

Problem	Probable Cause	Solution
Signal is high, control have saturated signal	Control reconstituted with less volume than required	Reconstitute new lyophilized control with the correct volume of solution recommended in the protocol.
	Control/samples, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain OD values greater than highest control point	Samples may require dilution and analysis.
High variation in samples and/or controls	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor control signal	The control was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Control was inappropriately stored	Store the reconstituted controled stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

Notes

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

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