



LAG3/MHC II BINDING ASSAY KITS

PROTOCOL

Part # 64ICP03PEG & 64ICP03PEH

Test size: 500 tests (64ICP03PEG), 10,000 tests (64ICP03PEH) - assay volume: 20 μ L

Revision: 03 (July 2017)

Store at: -60°C or below

For research use only. Not for use in diagnostic procedures.

ASSAY PRINCIPLE

The HTRF LAG3/MHC II Binding Assay is designed to measure the interaction between MHC II and LAG3 proteins. Utilizing HTRF (Homogeneous Time-resolved Fluorescence) technology, the assay enables simple and rapid characterization of compound and antibody blockers in a high throughput format.

As shown in Figure 1, the interaction between Tag1-LAG3 and Tag2-MHC II is detected by using anti-Tag1-Terbium (HTRF donor) and anti-Tag2-XL665 (HTRF acceptor). When the donor and acceptor antibodies are brought into close proximity due to LAG3 and MHC II binding, excitation of the donor antibody triggers fluorescent resonance energy transfer (FRET) towards the acceptor antibody, which in turn emits specifically at 665 nm. This specific signal is directly proportional to the extent of LAG3/MHC II interaction. Thus, compound or antibody blocking LAG3/MHC II interaction will cause a reduction in HTRF signal.

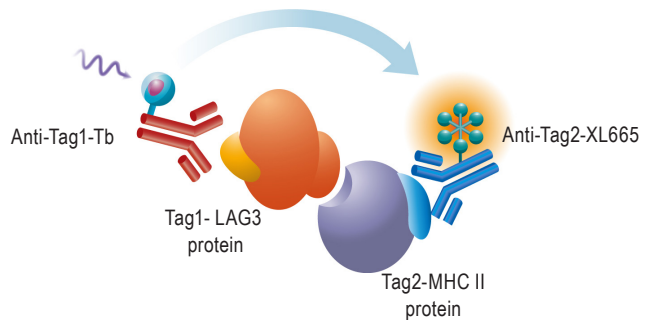
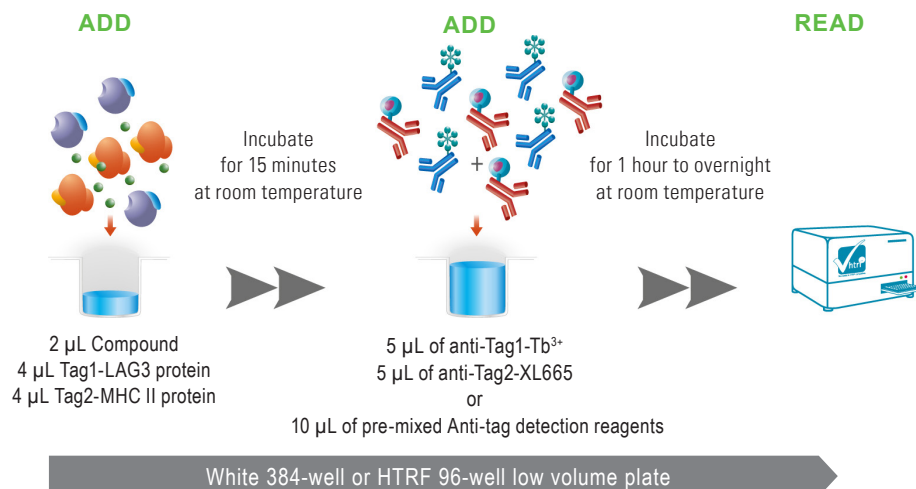


Figure 1: Principle of the HTRF MHC II/LAG3 assay.

PROTOCOL AT A GLANCE



Make sure to use the setup for Tb³⁺ Cryptate. For more information about set-up and compatible HTRF® readers, please visit our website at: <http://www.cisbio.com/readers>

MATERIALS:

KIT COMPONENTS	500 TESTS CAT # 64ICP03PEG	10,000 TESTS CAT # 64ICP03PEH
Tag1-LAG3* MW: 45.5 kDa	1 vial Frozen see concentration and volume on vial label	1 vial Frozen see concentration and volume on vial label
Tag2-MHC II* MW: 48 kDa	1 vial Frozen see concentration and volume on vial label	1 vial Frozen see concentration and volume on vial label
Anti-Tag1-Tb ³⁺	1 vial 25 µL 100 X Frozen	1 vial 0.5 mL 100 X Frozen
Anti-Tag2-XL665	1 vial 25 µL 100 X Frozen	1 vial 0.5 mL 100 X Frozen
Diluent	1 vial 20 mL Cat# 62DLBDDD ready-to-use	1 vial 200 mL Cat# 62DLBDDF ready-to-use
Detection Buffer	1 vial 10 mL Cat# 62DB2FDG (130 mL) ready-to-use	1 vial 130 mL Cat# 62DB2FDG (130 mL) ready-to-use

* The amounts of Tag1-LAG3 and Tag2-MHC II provided are sufficient for the validated amounts of tagged proteins suitable for compound inhibition study: 10 nM of LAG3 and 20 nM of MHC II in 20 µL final assay volume.

For reading, an HTRF®-Certified Reader is needed.

For HTRF microplate recommendations, please visit <http://www.cisbio.com/microplate-recommendations>

For a list of HTRF-compatible readers and setup recommendations, please visit <http://www.cisbio.com/readers>

STORAGE AND STABILITY

Store the kit at -60°C or below. Under appropriate storage conditions, reagents are stable until the expiry date indicated on the label.



Reagents

Once thawed, tagged LAG3 & MHC II stock solution may be frozen, and can be thawed only once. Once thawed (or reconstituted), anti-Tag solutions can be frozen once.

To avoid freeze/thaw cycles, it is recommended to dispense remaining stock solutions into disposable plastic vials for storage at -60°C or below.

Thawed diluent and detection buffer can be stored at 2-8°C on your premises.

REAGENT PREPARATION**BEFORE YOU BEGIN:**

- It is very important to prepare reagents in the specified buffers. The use of an incorrect diluent may affect reagent stability and assay results.
- Thaw the frozen reagents at room temperature.
- Before use, allow all reagents to warm up to room temperature then homogenize buffer and diluent. It is recommended to filter buffers before use.
- The tagged protein solutions must be prepared in individual vials - DO NOT premix tagged solutions prior to dispensing.
- The anti-Tag solutions must be prepared in individual vials and can be premix prior to dispensing.
- Compounds may be prepared in diluent. We recommend keeping DMSO below 0.5% during the assay (20 µL final volume).






TO PREPARE WORKING SOLUTIONS:

Take care to prepare stock and working solutions according to the directions for the kit size you have purchased.

500 TESTS	10,000 TESTS
Tag1-LAG3 protein Concentration and volume are indicated on the vial label	
Thaw the Tag1-LAG3 protein* solution. Prepare working solutions in diluent which have 5X the required final concentration for binding assay*. e.g. Prepare a 50 nM Tag1-LAG3 working solution for a final concentration of 10 nM Tag1-LAG3 (20 µL final volume).	
Tag2-MHC II protein Concentration and volume are indicated on the vial label	
Thaw the Tag2-MHC II protein* solution. Prepare working solutions in diluent which have 5X the required final concentration for binding assay*. e.g. Prepare a 100 nM Tag2-MHC II working solution for a final concentration of 20 nM Tag2-MHC II (20 µL final volume).	
Anti-Tag1-Tb	
Thaw the anti-Tag1-Tb ³⁺ solution. This 100 X stock solution can be frozen and stored at -60°C or below. Dilute 100-fold the 100 X anti-Tag1-Tb ³⁺ stock solution with detection buffer.	
e.g. 25 µL of thawed anti-Tag1-Tb ³⁺ stock solution + 2475 µL of detection buffer.	e.g. 0.5 mL of thawed anti-Tag1-Tb ³⁺ stock solution + 49.5 mL of detection buffer.
Anti-Tag2-XL665	
Thaw the anti-Tag2-XL665 solution. This 100 X stock solution can be frozen and stored at -60°C or below. Dilute 100-fold the 100 X anti-Tag2-XL665 stock solution with detection buffer.	
e.g. 25 µL of thawed anti-Tag2-XL665 stock solution + 2475 µL of detection buffer.	e.g. 0.5 mL of reconstituted anti-Tag2-XL665 stock solution + 49.5 mL of detection buffer.

*Titration of Tag1-LAG3 or Tag2-MHC II can be performed if necessary.

ASSAY PROTOCOL

Step 1		Dispense 2 µL of compound/antibody or diluent 4 µL of Tag1-LAG3 protein 4 µL of Tag2-MHC II protein.
Step 2		Incubate for 15 minutes at room temperature.
Step 3		Dispense 10 µL of pre-mixed anti-Tag1-Tb ³⁺ and anti-Tag2-XL665.
Step 4		Seal the plate and incubate for 1 hour to overnight at room temperature.
Step 5		Remove the plate sealer and read on an HTRF® compatible reader.

STANDARD PROTOCOL FOR INHIBITORY ASSAY IN 20 µL FINAL VOLUME

	Inhibitor	Tag1-LAG3	Tag2-MHC II	Anti-Tag1-Cryptate	Anti-Tag2-XL665	Diluent	Detection buffer
Sample	2 µL	4 µL	4 µL	5 µL	5 µL		
Positive control		4 µL	4 µL	5 µL	5 µL	2 µL	
Negative control			4 µL	5 µL	5 µL	6 µL	
Cryptate control				5 µL		10 µL	5 µL
Buffer control						10 µL	10 µL

EXAMPLE OF PLATE MAP

	1	2	3	4	5	6
A	Buffer control: 10 µL diluent 10 µL detection buffer	Repeat Well A1	Repeat Well A1	Compound...: 2 µL compound... 4 µL Tag1-LAG3 4 µL Tag2-MHC II 10 µL pre-mix anti-Tag reagents	Repeat Well A4	Repeat Well A4
B	Cryptate control: 10 µL diluent 5 µL detection buffer 5 µL anti-Tag1-Tb	Repeat Well B1	Repeat Well B1	Compound...: 2 µL compound... 4 µL Tag1-LAG3 4 µL Tag2-MHC II 10 µL pre-mix anti-Tag reagents	Repeat Well B4	Repeat Well B4
C	Negative control: 6 µL diluent 4 µL Tag2-MHC II 10 µL pre-mix anti-Tag reagents	Repeat Well C1	Repeat Well C1	Compound...: 2 µL compound... 4 µL Tag1-LAG3 4 µL Tag2-MHC II 10 µL pre-mix anti-Tag reagents	Repeat Well C4	Repeat Well C4
D	Positive control: 2 µL diluent 4 µL Tag1-LAG3 4 µL Tag2-MHC II 10 µL pre-mix anti-Tag reagents	Repeat Well D1	Repeat Well D1	Compound...: 2 µL compound... 4 µL Tag1-LAG3 4 µL Tag2-MHC II 10 µL pre-mix anti-Tag reagents	Repeat Well D4	Repeat Well D4
E	Compound 1: 2 µL compound 1 4 µL Tag1-LAG3 4 µL Tag2-MHC II 10 µL pre-mix anti-Tag reagents	Repeat Well E1	Repeat Well E1	Compound...:	Repeat Well E4	Repeat Well E4
F	Compound 2: 2 µL compound 2 4 µL Tag1-LAG3 4 µL Tag2-MHC II 10 µL pre-mix anti-Tag reagents	Repeat Well F1	Repeat Well F1	Compound...:	Repeat Well F4	Repeat Well F4
G	Compound...: 2 µL compound... 4 µL Tag1-LAG3 4 µL Tag2-MHC II 10 µL pre-mix anti-Tag reagents	Repeat Well G1	Repeat Well G1	Compound...:	Repeat Well G4	Repeat Well G4
H	Compound...: 2 µL compound... 4 µL Tag1-LAG3 4 µL Tag2-MHC II 10 µL pre-mix anti-Tag reagents	Repeat Well H1	Repeat Well H1			

DATA REDUCTION & INTERPRETATION

1. Calculate the ratio of the acceptor and donor emission signals for each individual well.

$$\text{Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10^4$$

2. Calculate the % CVs. The mean and standard deviation can then be worked out from ratio replicates.

$$\text{CV (\%)} = \frac{\text{Standard deviation}}{\text{Mean Ratio}} \times 100$$

For more information about data reduction, please visit <http://www.cisbio.com/data-reduction>

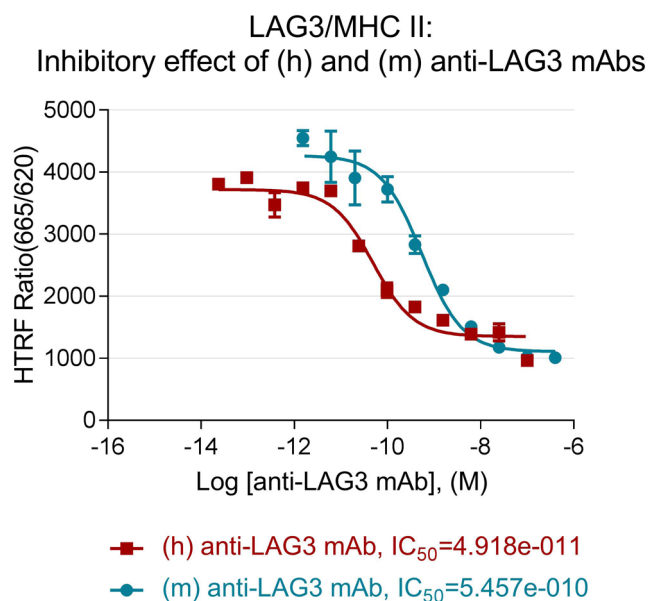
RESULTS

The data shown below must not be substituted for the data obtained in the laboratory, and should be considered only as an example.

The inhibitory effect of Human anti-LAG3 was tested at 10 nM LAG3 and 20 nM MHC II.

Readouts on PHERAstar FS with a flash lamp.

Note that results may vary from one HTRF® compatible reader to another.



This product contains material of biological origin. Use for research purposes only. Do not use in humans or for diagnostic purposes. The purchaser assumes all risk and responsibility concerning reception, handling and storage. The use of the cell line will be done with appropriate safety and handling precautions to minimize health and environmental impact. Remaining disclaimer.

Copyright 2017 Cisbio, France. All rights reserved. HTRF, Tag-lite, EPIgeneous and the HTRF logo are trademarks or registered trademarks of Cisbio. All other trademarks are the property of their respective owners.

FOR MORE INFORMATION

Europe and other countries +33(0)466-796-705 U.S. and Canada 1-888-963-4567 China +86-21-5018-9880
Japan +81-(0)43-306-8712 Visit www.cisbio.com to find a list of our regional distributors