



EP155-EN.01

Integrin α 4 β 7: MAdCAM-1 [Biotinylated] Inhibitor Screening ELISA Kit

Pack Size: 96 tests

Catalog Number: EP-155

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use In Diagnostic Or Therapeutic Procedures

INTENDED USE

This kit is designed for screening of inhibitors of binding between human Integrin $\alpha 4 \beta 7$ and human MAdCAM-1. It is intended for research use only (RUO).

PRINCIPLE OF THE ASSAY

This inhibitor screening ELISA kit is designed to facilitate the identification and characterization of new Integrin $\alpha 4 \beta 7$ pathway inhibitors. The assay takes advantage of our in house-developed binding of biotinylated human MAdCAM-1 to immobilized human Integrin $\alpha 4 \beta 7$ in a functional ELISA assay and employs a simple colorimetric ELISA platform. Briefly, we provide you with a human Biotinylated MAdCAM-1 protein, a human Integrin $\alpha 4 \beta 7$ protein, an anti-Integrin $\alpha 4 \beta 7$ neutralizing antibody (as method verified Std.), and Streptavidin-HRP reagent. Your experiment will include 4 simple steps:

- 1) Coat the plate with human Integrin $\alpha 4 \beta 7$.
- 2) Add your molecule of interest to the tests.
- 3) Add human MAdCAM-1-Biotin to bind the coated human Integrin $\alpha 4 \beta 7$.
- 4) Add Streptavidin-HRP followed by TMB or other colorimetric HRP substrate.

Finally, the half maximal inhibitory concentration (IC₅₀) of your compound to Integrin $\alpha 4 \beta 7$: MAdCAM-1 binding will be determined by comparing OD readings among different experimental groups.

MATERIALS PROVIDED

TABLE 1. MATERIALS PROVIDED (pls modify according to COA)

Catalog	Components	Size (96 tests)	Format	Storage
EP155-C01	High-bind Plate	1 plate	Solid	2-8°C
EP155-C02	Human Integrin $\alpha 4 \beta 7$	25 μ g	Powder	2-8°C
EP155-C03	Biotinylated Human MAdCAM-1	10 μ g	Powder	2-8°C
EP155-C04	Anti- Integrin $\alpha 4 \beta 7$ Neutralizing Antibody	20 μ g	Powder	2-8°C
EP155-C05	Streptavidin-HRP	5 μ g	Powder	2-8°C, avoid light
EP155-C06	Coating Buffer	12 mL	Liquid	2-8°C
EP155-C07	10xWashing Buffer	50 mL	Liquid	2-8°C
EP155-C08	Blocking Buffer	50 mL	Liquid	2-8°C
EP155-C09	Substrate Solution	12 mL	Liquid	2-8°C, avoid light
EP155-C10	Stop Solution	7 mL	Liquid	2-8°C
EP155-C10	1mol/L CaCl ₂	1.5 mL	Liquid	2-8°C
EP155-C10	1mol/L MgCl ₂	1.5 mL	Liquid	2-8°C
EP155-C10	1mol/L MnCl ₂	1.5 mL	Liquid	2-8°C

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or dual wavelength microplate reader with 450 nm/630nm filter ;

Centrifuge ;

37 °C Incubator ;

Single channel or multichannel pipettes with 10 μ L, 200 μ L and 1000 μ L precision ;

10 μ L, 200 μ L and 1000 μ L pipette tips ;

Test Tubes ;

Graduated cylinder ;

Deionized or distilled water for dilution ;

STORAGE AND VALIDITY INSTRUCTIONS

Unopened kit should be stored at 2°C -8°C upon receiving. Find the expiration date on the outside packaging and do not use reagents past their expiration date.

The kit should be stored as TABLE 1 after the reconstitution of lyophilized materials. The shelf life is 30 days from the date of opening.

Note:

a. Do not use reagents past their expiration date.

b. Find the expiration date on the outside packaging.

REAGENT PREPARATION

1. Restore all reagents and samples to room temperature (20-25°C) before use.

2. Reconstitute the provided lyophilized materials to stock solutions with sterile deionized water as recommended in Tab.2, Solubilize for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking or vortex. The reconstituted stock solutions should be stored at -70°C. **Avoid freeze-thaw cycles.**

Note: Streptavidin-HRP stock solution should be protected from light.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

Catalog	Components	Amount	Stock Solution Con.	Reconstitution Buffer and Vol.
EP155-C02	Human Integrin $\alpha 4 \beta 7$	25 μg	100 $\mu\text{g}/\text{mL}$	250 μL , water
EP155-C03	Biotinylated Human MAdCAM-1	10 μg	100 $\mu\text{g}/\text{mL}$	100 μL , water
EP155-C04	Anti- Integrin $\alpha 4 \beta 7$ Neutralizing Antibody	20 μg	100 $\mu\text{g}/\text{mL}$	200 μL , water
EP155-C05	Streptavidin-HRP	5 μg	50 $\mu\text{g}/\text{mL}$	100 μL , water

RECOMMENDED PROTOCOL

1. Working solution preparation

1.1 Preparation of Coating Buffer:

Add 0.012 mL CaCl_2 and 0.012 mL MgCl_2 to 12 mL Coating Buffer (EP155-C06).

1.2 Preparation of 1×Washing Buffer:

Add 50 mL 10×Washing Buffer and 0.5 mL 1mol/L MnCl₂ to 449.5 mL ultrapure water/deionized water

1.3 Preparation of Blocking Buffer:

Add 0.05 mL 1mol/L MnCl₂ to 49.95 mL Blocking Buffer (EP155-C08).

1.4 Preparation of Dilution Buffer:

Add 10 mL Blocking Buffer Contained 1mmol/L MnCl₂ to 30 mL 1×Washing Buffer.

2. Coating

1) Dilute Human Integrin $\alpha 4 \beta 7$ stock solution (100 $\mu\text{g}/\text{mL}$) to 1.8 $\mu\text{g}/\text{mL}$ with Coating Buffer Contained 1mmol/L CaCl₂ and 1mmol/L MgCl₂ to make Human Integrin $\alpha 4 \beta 7$ working solution.

2) Add 100 μL of Human Integrin $\alpha 4 \beta 7$ working solution (1.8 $\mu\text{g}/\text{mL}$) to each well and leave a couple of wells uncoated for No-Coating Control, seal the plate with microplate sealing film and incubate overnight (or 16 hours) at 4°C.

3. Washing

Remove the remaining solution by aspiration, add 300 μL of 1×Washing Buffer to each well, gently tap the plate for 1 minute, remove any remaining 1×Washing Buffer by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the washing step above for three times.

Note: For best results, the complete removal of the Human Integrin $\alpha 4 \beta 7$ solution is essential. The use of a manifold dispenser or an auto-washer may be necessary.

4. Blocking

Add 300 μL Blocking Buffer to each well, seal the plate with microplate sealing film and incubate at 37°C for 1.5 hours.

5. Washing

Repeat step 3. At the same time, you can start to prepare your samples.

6. Add Samples

1) Make serial dilution of the samples as appropriate.

2) If you intend to use the provided Anti-Integrin $\alpha 4 \beta 7$ Neutralizing Antibody as a reference (Std.), you may dilute the antibody as recommended in Figure 1.

3) Add 50 μL of sample solution to each well according to our recommendation (Figure 2) or your own plate setup.

4) For No-Coating Control wells, please add 50 μL Dilution Buffer.

7. Binding

1) Dilute Biotinylated Human MAdCAM-1 stock solution (100 $\mu\text{g}/\text{mL}$) to 0.1 $\mu\text{g}/\text{mL}$ with Dilution Buffer to make Biotinylated Human MAdCAM-1 working solution.

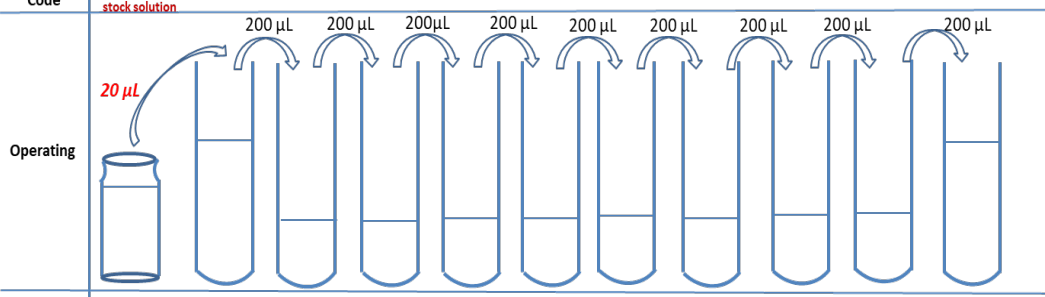
2) For No-binding control wells, please add 50 μL Dilution Buffer.

3) For all other wells, please add 50 μL Biotinylated Human MAdCAM-1 working solution to the wells and mix the samples by gently tapping the plate. Seal the plate with microplate sealing film and incubate at 37°C for 1 hour.

Note: The working solution should be prepared immediately before use and should not be stored.

FIG.1 PREPARATION OF 1:1 SERIAL DILUTIONS OF THE Anti-Integrin $\alpha 4 \beta 7$ Neutralizing Antibody

FIG.2 PLATE LAYOUT

Tubes/ Solution Code	Anti-Integrin $\alpha 4$ $\beta 7$ Neutralizing Antibody stock solution	Std.-1	Std.-2	Std.-3	Std.-4	Std.-5	Std.-6	Std.-7	Std.-8	Std.-9	Std.-10
Operating		200 μL	200 μL	200 μL	200 μL	200 μL	200 μL	200 μL	200 μL	200 μL	200 μL
Solution Con.	100 $\mu\text{g}/\text{mL}$	5 $\mu\text{g}/\text{mL}$	2.5 $\mu\text{g}/\text{mL}$	1.25 $\mu\text{g}/\text{mL}$	0.625 $\mu\text{g}/\text{mL}$	0.312 $\mu\text{g}/\text{mL}$	0.156 $\mu\text{g}/\text{mL}$	0.078 $\mu\text{g}/\text{mL}$	0.039 $\mu\text{g}/\text{mL}$	0.0195 $\mu\text{g}/\text{mL}$	0.01 $\mu\text{g}/\text{mL}$
Dilution Buffer Vol.		380 μL	200 μL	200 μL	200 μL	200 μL	200 μL	200 μL	200 μL	200 μL	200 μL

8. Washing

Repeat step 3.

9. Add Streptavidin-HRP

1) Dilute Streptavidin-HRP stock solution (50 µg/mL) to 0.1 µg/mL with Dilution Buffer to make Streptavidin-HRP working solution.

2) For all wells, add 100 µL Streptavidin-HRP working solution, seal the plate with microplate sealing film and incubate at 37°C for 1 hour, avoid light.

10. Washing

Repeat step 3.

11. Substrate Reaction

Add 100 µL **Substrate Solution** to each well. Seal the plate with microplate sealing film and incubate at 37°C for 20 minutes. Avoid light.

12. Termination

Add 50 µL **Stop Solution** to each well, and gently shake the plate to allow thorough mixing.

Note: the color in the wells should change from blue to yellow.

13. Data Recording

Read the absorbance at 450 nm using UV/Vis microplate spectrophotometer.

Note: Subtracting the value read at OD_{450nm} with OD_{630nm} can be used to reduce the background noise.

SIMPLIFIED PROTOCOL

TABLE. 3 ASSAY PROTOCOL

Steps Code	Steps	Reagents & Instruments	Reaction Conditions	Samples	No-binding Ctrl.	No-coating Ctrl.	Positive Ctrl.
1	Working fluid preparation	N/A	N/A	N/A	N/A	N/A	N/A
2	Coating	Human Integrin $\alpha 4 \beta 7$ Working Solution	4°C for overnight	100 µL	100 µL	—	100 µL
3	Washing	1X Wash Buffer	Wash for 3 times	300 µL	300 µL	300 µL	300 µL

4	Blocking	Blocking Buffer	37°C for 1.5 hours	300 µL	300 µL	300 µL	300 µL	
5	Washing	1XWash Buffer	Wash for 3 times	300 µL	300 µL	300 µL	300 µL	
6	Add Samples	Samples	—	50 µL	—	—	—	
		Dilution Buffer		—	50 µL	50 µL	50 µL	
7	Binding	Biotinylated Human MAdCAM-1 Working Solution		Mix by gentle tapping, incubate at 37°C for 1 hours	50 µL	—	50 µL	50 µL
		Dilution Buffer			—	50 µL	—	—
8	Washing	1XWash Buffer	Wash for 3 times		300 µL	300 µL	300 µL	300 µL
9	Streptavidin-HRP	Streptavidin-HRP Working Solution	37°C for 1 hours		100 µL	100 µL	100 µL	100 µL
10	Washing	1XWash Buffer	Wash for 3 times	300 µL	300 µL	300 µL	300 µL	
11	Substrate Reaction	Substrate Solution	37°C for 20 minutes	100 µL	100 µL	100 µL	100 µL	
12	Termination	Stop Solution	Mix by gentle tapping	50 µL	50 µL	50 µL	50 µL	
13	Data Recording	UV/Vis spectrophotometer	Measure absorbance at 450 nm, with the correction wavelength set at 630 nm					

Note for TAB. 3:

- 1) *Samples: Your samples of interest.*
- 2) *No-binding Ctrl.: Reaction without Biotinylated Human MAdCAM-1 added. The absorbance should be around 0.05(< 0.1) at 450 nm.*
- 3) *No-coating Ctrl.: Reaction without Human Integrin $\alpha 4 \beta 7$ coated on the wells. The absorbance should be around 0.05(< 0.1) at 450 nm.*
- 4) *Positive Ctrl.: Determined the max value in 450nm absorbance, when out of inhibitors.*
- 5) *It is recommended that all samples, controls and standards should be done in duplicates.*

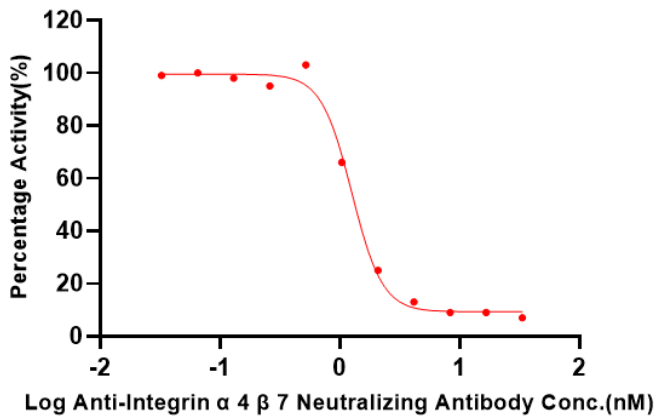
PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. This kit should be used according to the provided instructions.
3. Do not mix reagents from different lots.
4. All reagents should be balanced to room temperature (20°C-25°C) before use.
5. This kit should be stored at 2°C-8°C.
6. Please prepare the working solution of each component according to the needs of the experiment. Except for 1x Washing Buffer, all prepared working solution is for one-time use and cannot be stored.

METHOD VERIFICATION

INHIBITION OF Integrin $\alpha 4 \beta 7$: MAdCAM-1 [Biotinylated] BINDING BY ANTI-Integrin $\alpha 4 \beta 7$ NEUTRALIZING ANTIBODY

Serial dilutions of Anti- Integrin $\alpha 4 \beta 7$ Neutralizing antibody (Catalog # EP155-C04) (1:1 serial dilution, from 5 $\mu\text{g/mL}$ to 0.0098 $\mu\text{g/mL}$) was added into Integrin $\alpha 4 \beta 7$: Biotinylated MAdCAM-1 binding reactions. The assay was performed according to the protocol described below. Background was subtracted from data points prior to log transformation and curve fitting.



Anti-Integrin $\alpha 4 \beta 7$ Neutralizing Antibody Conc. ($\mu\text{g/ml}$)	Anti-Integrin $\alpha 4 \beta 7$ Neutralizing Antibody Conc. (nM)	Mean Abs.(OD450)	Percentage Activity(%)
0	0.000	2.607	100%
0.010	0.065	2.615	100%
0.020	0.130	2.542	98%
0.039	0.260	2.466	95%
0.078	0.521	2.684	103%
0.156	1.042	1.724	66%
0.313	2.083	0.66	25%
0.625	4.167	0.345	13%
1.250	8.333	0.238	9%
2.50	16.667	0.24	9%
5.0	33.333	0.192	7%
No Coating		0.067	
No Binding		0.055	