HID5/Psoriasin/S100A7 ActivELISA™

For the Detection of Human HID5/Psoriasin/S100A7 in Tissue and body fluid

Catalog No.: IMK-514
Version 06 2008

2 x 96 well format

The IMK-514 HID5/Psoriasin/S100A7 ActivELISA™ Kit contains the HID5/Psoriasin/S100A7 **ActivELISA™ Module**

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TABLE OF CONTENTS

Background 3

Overview 3

Advantages 4

Experiment 5

Kit Components and Storage 6

Preparation of Reagents 6-7

HID5 ActivELISA™ Protocol 7-9

Product Citations 10

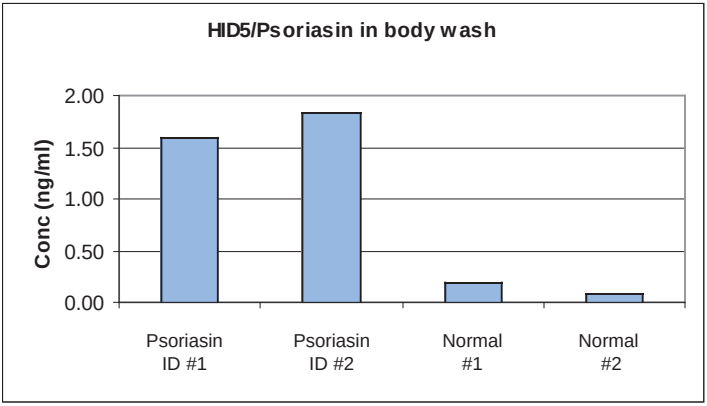
Troubleshooting 10

I. BACKGROUND

HID-5/Psoriasin was originally identified as an up-regulated protein in psoriatic keratinocytes (1). Subsequently, this protein was found to have elevated expression in abnormally differentiating primary keratinocytes and in various carcinomas, including ductal carcinoma *in situ* (2, 3). HID-5/Psoriasin may play a role in the establishment or maintenance of these pathologies, and may serve as a diagnostic marker of particular tumor types or abnormally proliferative epithelia.

II. OVERVIEW

This ELISA kit is suitable for the *in vitro* quantitation of HID5/Psoriasin protein from cell lysate and serum samples. The HID5/Psoriasin ActivELISA™ measures the soluble form of HID5 protein using a sandwich ELISA protocol. The protein is captured on the microtiter plate using an anti-HID5 pAb antibody. The amount of bound HID5/Psoriasin is then detected by adding an anti-HID5 monoclonal antibody followed by HRP conjugated secondary and using colorimetric detection. This ELISA kit provides quick, quantifiable results using a standard curve which is generated with the included recombinant HID5/Psorisin protein. The kit can detect nanogram quantities of HID5/Psoriasin protein in patient samples.



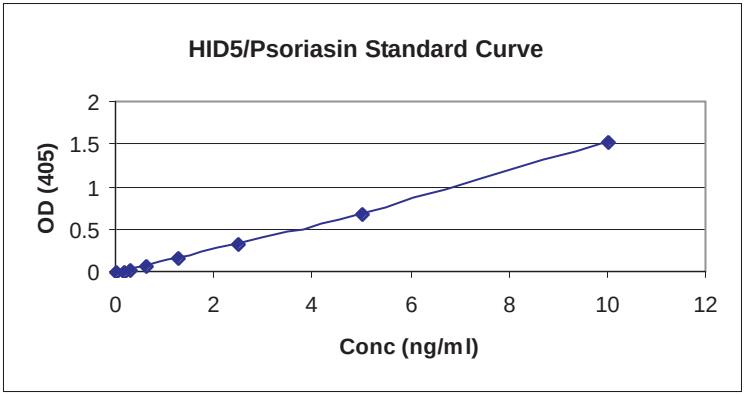
III. ADVANTAGES

- Contains reagents and protocol to prepare whole cell lysate, body fluides and tissue lysate.
- Multiple samples can be analyzed in a low-volume, high-throughput format.
- Full analysis complete in just hours.

IV. EXPERIMENT

EXAMPLE STANDARD

Following the provided protocol, HID5/Psoriasin/S100A7 standard was titrated to determine detectible levels.



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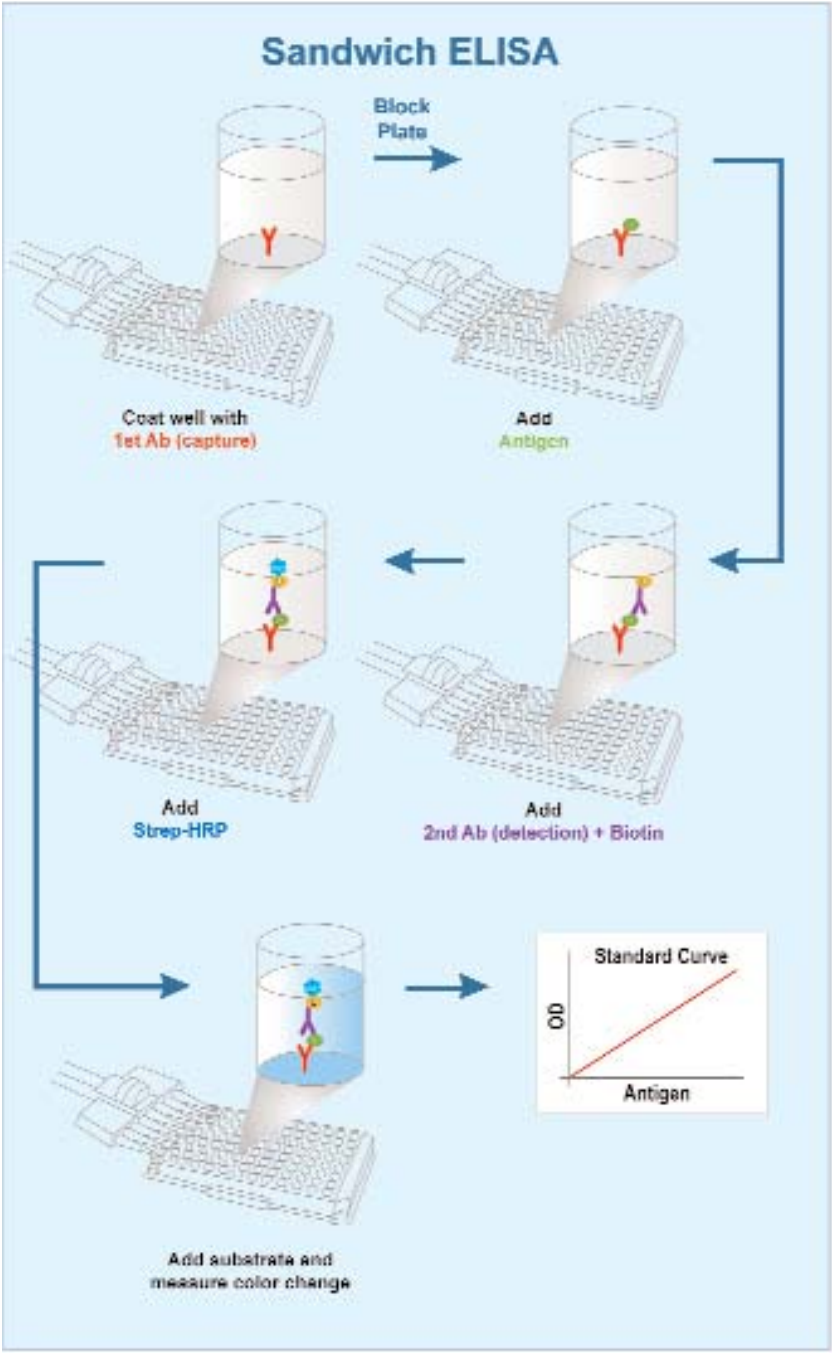
Sandwich ELISA
Block Plate
Coat well with 1st Ab (capture)
Add Antigen
Add 2nd Ab (detection) + Biotin
Add Strep-HRP
Add substrate and measure color change
Standard Curve
OD
Antigen

V. KIT COMPONENTS AND STORAGE

REAGENTS (4°C STORAGE)		
IMK-514-01	**Capture Antibody***	2 x 100 µl
IMK-514-03	**Detecting Antibody***	2 x 100 µl
KC-104	**Coating Buffer**	2 x 10 ml
KC-100	**BSA**	2 x 0.5 g
KC-101	**20X Wash Buffer**	50 ml
KC-143	**HRP-Conjugated Streptavidin**	10 µl
KC-106	**HRP Substrate**	2 x 10 ml
REAGENTS (-80°C, STORAGE--NON FROST-FREE FREEZER)		
IMK-514-02	**Recombinant HID5/Psoriasin/S100A7 Standard** 2 vials, 50 ul per vial, 100 ng/ml	
ADDITIONAL ITEMS INCLUDED		
ELISA Plates		2
IMK-514 Manual		1

** Contains 0.02 % Sodium azide. Sodium azide is highly toxic.*

Additional items required for the ELISA (not included in the *IMK-514 ActivELISA*):

- Distilled water
- 96-well ELISA plate reader

VI. PREPARATION OF REAGENTS: HID5/PSORIASIN/S100A7 ActivELISA™

NOTE: *The included buffers and reagents are optimized for use with this kit. Substitution with other reagents is not recommended and may not give optimal results.*

1X Wash Buffer: Prepare 1X Wash Buffer by diluting 20X Wash Buffer (KC-101) in distilled water. The diluted Wash Buffer may be stored at 4°C, however we recommend preparing fresh 1X Wash Buffer for each experiment.

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Blocking Buffer: Dissolve 0.5 g BSA (KC-100) in 50 ml of 1X Wash Buffer in a sterile bottle.

NOTE: *To prevent microbial growth keep Blocking Buffer and 1X Wash Buffer stored at 4°C when not in use and reduce exposure to contaminants. Sodium azide at a final concentration of 0.02% may be added to prevent bacterial growth. Two aliquots of BSA have been included. Each aliquot contains sufficient BSA for one plate. Prepare Blocking Buffer fresh prior to experiment.*
Sodium azide is an inhibitor of HRP. Blocking buffer that contains sodium azide can not be used to dilute the HRP secondary antibody.

VII. HID5/Psoriasin/S100A7 ActivELISA™ PROTOCOL

This kit allows for the quantitative measurement of HID5/Psoriasin/S100A7 activation in a 96-well microtiter format. All 96-wells may be used at one time or you may only use the wells as required by your experimental design. Use of duplicate wells for each time point are recommended to obtain accurate results.

APPROPRIATE CONTROLS TO INCLUDE

Following is a list of suggested controls to include with each analysis:

1. No capture antibody added to well
2. No lysate added to well
3. No capture antibody or lysate added to well
4. Positive control: use a cell line or tissue known to constitutively express HID5/Psoriasin/S100A7 or patient body fluid
5. Negative control: use a cell line or tissue known to not express HID5/Psoriasin/S100A7

ELISA PROTOCOL

1. **Coating:** Dilute 100 µl of Capture Antibody (IMK-514-01) in 10 ml (final volume) of Coating Buffer (KC-104). Pipet 100 µl of diluted antibody into each well (**A1** through **H1** and **A2** through **H2 f**or the standard and any of columns 3 through 12 for your samples), cover and incubate the plate overnight (12-24 h) at 4°C. Wash the coated wells twice with 300 µl of 1X Wash Buffer.

2. **Blocking:** Add 200 µl of prepared Blocking Buffer to each well to block the remaining reactive surface. Incubating for 30 min to 1 h at RT.

3. **Prepare HID5 Standard Curve:** Dilution of standard: Add 450 ul of Blocking Buffer to 50 ul of stock Recombinant HID5 to make a working solution of 10 ng/ml. Set up a standard curve in duplicate using the following concentrations: 10.0, 5.0, 2.5, 1.25, 0.62, 0.31, 0.16 and 0.0 (blank) ng/ml. To obtain an accurate result, we suggest using the test samples in duplicate. (see Table 1 for suggested layout).

   - Remove Blocking Buffer from wells by flicking into an appropriate waste container and gently tapping the plate face-down on paper towels. Replace with 100 µl of fresh prepared Blocking Buffer in each well **B1** through **H1** and **B2** through **H2** for the standard.

   - Pipette 200 µl diluted Recombinant HID5/Psoriasin/S100A7 Standard (10.0 ng/ml) into wells **A1** and **A2**. Transfer 100 µl from wells **A1** and **A2** in to wells **B1** and **B2**.
   - Mix wells **B1** and **B2** by pipetting.
   - Transfer 100 µl from well **B1** to **C1** and **B2** to **C2**.
   - Continue this serial dilution process to wells **G1** and **G2**. After mixing, discard 100 µl of solution from wells **G1** and **G2**.
   - Do not add standard to wells **H1** and **H2**. These will serve as blanks.

Table 1. Set up of a 96-well microtiter plate.

	Standard	Standard	Your Samples									
	1	2	3	4	5	6	7	8	9	10	11	12
A	10.0 ng/ml	10.0 ng/ml	-	-	-	-	-	-	-	-	-	-
B	5.0 ng/ml	5.0 ng/ml	-	-	-	-	-	-	-	-	-	-
C	2.5 ng/ml	2.5 ng/ml	-	-	-	-	-	-	-	-	-	-
D	1.25 ng/ml	1.25 ng/ml	-	-	-	-	-	-	-	-	-	-
E	0.625 ng/ml	0.625 ng/ml	-	-	-	-	-	-	-	-	-	-
F	0.31 ng/ml	0.31 ng/ml	-	-	-	-	-	-	-	-	-	-
G	0.16 ng/ml	0.16 ng/ml	-	-	-	-	-	-	-	-	-	-
H	Blank	Blank	-	-	-	-	-	-	-	-	-	-

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4. **Samples:** Pipet 100 µl of positive and negative controls and 100 µl test samples into the appropriate wells*. Incubate plate at 4ºC overnight or 4 h at RT. Samples may be diluted or serially diluted using Blocking Buffer. *Users need to empirically determine the optimal concentrations of their test samples so that the readings approximately fall within the curve of the protein standard.

5. **Washing:** Remove samples and control lysates and wash 4X with 300 µl of 1X Wash Buffer. Tap plate several times upside down to remove residual Wash Buffer after final wash.

6. **Detecting Antibody:** Dilute 100 µl of Detecting Antibody (IMK-514-03) in 10 ml of Blocking Buffer and add 100 µl diluted Detecting Antibody to each well. Incubate for 1 h at RT.

7. **Washing:** Remove antibody solution and wash wells 4X with 300 µl of 1X Wash Buffer. Tap plate upside down to remove residual Wash Buffer after final wash.

8. **Secondary Antibody:** Dilute 4 µl of HRP-Conjugated Streptavidin (KC-143) in 10 ml of Blocking Buffer without sodium azide (for one plate). Add 100 µl of diluted secondary antibody to each well and incubate for 1 h at RT.

9. **Washing:** Remove the secondary antibody and wash thoroughly (5X) with 300 µl of Wash Buffer letting the solution sit briefly between each wash. This ensures a thorough wash and lower background. Tap plate upside down several times to remove any residual Wash Buffer.

10. **HRP Substrate**: Add 100 µl of HRP Substrate to each well. Incubate the plate at RT for 30 min. Read the color development at 405-410 nm.

NOTE: *Incubation time with HRP Substrate may be increased or decreased depending on the concentration of samples.*

VIII. CITATIONS

1. Madsen, P., Rasmussen, H., et. al. Molecular cloning, occurrence, and expression of a novel partially secreted protein 'psoriasin' that is highly up-regulated in psoriatic skin", *J. Investig. Derm*., 97:701-712 (1991).

2. Watson, P., Leygue, E., et. al. Psoriasin (S100A7), Int. J. Biochem. *Cell. Biol.*, 30: 567-571(1998).

3. Enerback, C., Porter, D., Psoriasin expression in mammary epithelial cells *in vitro* and *in vivo*. *Cancer Res.*, 62:43-47 (2002).

IX. TROUBLESHOOTING

Problem	Possible Cause	Suggestion
No signal	Failure to add all components	Prepare a check-list and add the components in the correct order. Confirm sodium azide is not present in HRP 2° antibody buffer
Low signal	Not enough sample per well	Check the protein concentration. A greater concentration of sample may be needed.
High background	Improper blocking Wells are not washed enough	Incubate with blocking buffer as recommended in the manual. Wash plates thoroughly after incubation with secondary antibody. Soak wells 20-30 sec between each wash.

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