PRODUCT INFORMATION & MANUAL

Human PD-L1/B7-H1 Valukine™ ELISA

VAL117

For the quantitative determination of natural and recombinant
human programmed cell death-Ligand 1(PD-L1/B7-H1) concentrations

For research use only.
Not for diagnostic or therapeutic procedures.

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Please refer to the kit label for expiry date.
Novus kits are guaranteed for 3 months from date of receipt

Version202006.3

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I. BACKGROUND

Programmed cell death-Ligand 1 (PD-L1), also known as B7-H1 and CD274, is an approximately 65 kDa transmembrane glycoprotein in the B7 family of immune regulatory molecules (1). PD-L1 is expressed on inflammatory-activated immune cells including macrophages, T cells, and B cells (2-5), keratinocytes (6, 7), endothelial and intestinal epithelial cells (6, 8), as well as a variety of carcinomas and melanoma (9, 10). PD-L1 binds to PD-1 and T cell B7-1/CD80 (5, 6, 10-13). It suppresses T cell activation and proliferation (3, 6, 12, 14) and induces the apoptosis of activated T cells (9). It plays a role in the development of immune tolerance by promoting T cell anergy (5, 12) and enhancing regulatory T cell development (14). PD-L1 favors the development of anti-inflammatory IL-10 and IL-22 producing dendritic cells (3, 8) and inhibits the development of Th17 cells (14). In cancer, PD-L1 provides resistance to T cell mediated lysis, enhances epithelial to mesenchymal transition (EMT), and enhances the tumorigenic function of Th22 cells (4, 7, 10, 13). A soluble form of PD-L1 is elevated in the plasma of cancer patients and in the cerebrospinal fluid in glioma (15-20). It can be released by mature dendritic cells and retains the ability to bind PD-1 and induce T cell apoptosis (19, 21, 22).

II. OVERVIEW

A. PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for human PD-L1/B7-H1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any PD-L1/B7-H1 present is bound by the immobilized antibody. After washing away any unbound substances, a biotin-linked detect antibody specific for human PD-L1/B7-H1 are pipetted into the wells. After washing away any unbound substances, streptavidin-HRP is added. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of PD-L1/B7-H1 bound in the initial step. The color development is stopped, and the intensity of the color is measured.

B. LIMITATIONS OF THE PROCEDURE

- **FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.**
- This kit is suitable for cell culture supernate, serum and plasma.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with Calibrator Diluent and repeat the assay.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.

III. ADVANTAGES

A. PRECISION

Intra-assay Precision (Precision within an assay)

Three samples were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)

Three samples were tested in twenty separate assays to assess inter-assay precision.

	Intra-assay Precision			**Inter-assay Precision**		
Sample	1	2	3	1	2	3
Mean (pg/mL)	590.7	1596.9	5107.3	596.8	1663.3	5850.1
Standard Deviation	18.2	95.1	409.8	18.5	64.7	555.5
CV%	3.1	6.0	8.0	3.1	3.9	9.5

B. RECOVERY

The recovery of human PD-L1/B7-H1 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	**Average % Recovery**	**Range**
Cell culture media (n=4)	97	83 - 107%
Serum (n=5)	117	114 - 119%
Plasma (n=4)	96	85 - 109%

C. SENSITIVITY

The minimum detectable dose (MDD) of human PD-L1/B7-H1 is typically less than 11.5 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

D. CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human PD-L1/B7-H1 produced at R&D Systems®.

E. LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of human PD-L1/B7-H1 in various matrices and diluted with Diluent 1× to produce samples with values within the dynamic range of the assay.

Dilution		Cell culture media (n=4)	Serum (n=5)	Plasma (n=4)
1:2	Average % of Expected	92	106	100
	Range (%)	91 - 93	97 - 113	83 - 110
1:4	Average % of Expected	94	107	96
	Range (%)	92 - 96	100 - 115	85 - 112
1:8	Average % of Expected	95	110	98
	Range (%)	93 - 98	105 - 118	81 - 117
1:16	Average % of Expected	100	114	101
	Range (%)	95 - 105	107 - 123	88 - 124

F. SAMPLE VALUES

Serum - Five human serum samples were evaluated for the presence of PD-L1/B7-H1 in this assay. All samples measured ranged from 392 to 562 pg/mL with an average of 486.2 pg/mL.

Plasma - Four human plasma samples were evaluated for the presence of human PD-L1/B7-H1 in this assay. All samples measured below the lowest standard, 156.3pg/mL.

G. SPECIFICITY

This assay recognizes both natural and recombinant human PD-L1/B7-H1. The following factors prepared at 100 ng/mL were assayed and exhibited no cross-reactivity or interference.

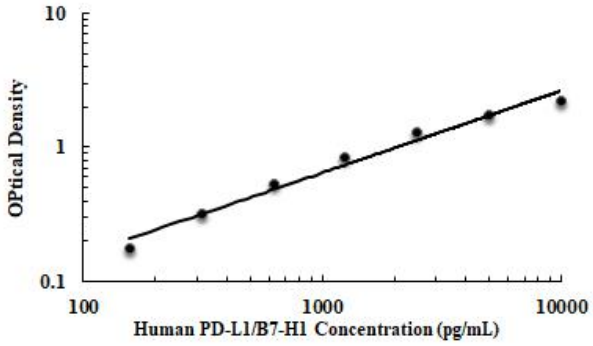
Recombinant human	**Other recombinants**
B7-1/Fc Chimera	mouse B7-H1 Fc Chimera
B7-2 Fc Chimera	Rat B7-1 Fc Chimera
B7-H2 Fc Chimera	
B7-H3	
B7-H4	
B7-H6 Fc Chimera	
PD-L2 Fc Chimera	

Recombinant human PD-1/Fc Chimera does not cross-react in this assay but does interfere at concentrations > 50 ng/mL.

IV. EXPERIMENT

EXAMPLE STANDARD

The standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



pg/mL	OD	Average	Corrected
0	0.075 0.067	0.071	-
156.25	0.250 0.248	0.249	0.178
312.5	0.385 0.394	0.390	0.319
625	0.596 0.604	0.600	0.529
1250	0.906 0.899	0.903	0.832
2500	1.354 1.357	1.356	1.285
5000	1.806 1.809	1.808	1.737
10000	2.317 2.284	2.301	2.230

V. KIT COMPONENTS AND STORAGE

A. MATERIALS PROVIDED

Parts	Description	Size
Human PD-L1/B7-H1 Microplate	96 well polystyrene microplate (12 strips of 8 wells) coated with a mouse antibody against human PD-L1/B7-H1.	1 plate
Human PD-L1/B7-H1 Standard	Recombinant human PD-L1/B7-H1 in a buffered protein base; lyophilized. Refer to the vial label for reconstitution volume.	2 vials
Human PD-L1/B7-H1 Detection antibody	Biotinylated human PD-L1/B7-H1 polyclonal antibody，lyophilized. Refer to the vial label for reconstitution volume.	1 vial
Calibrator Diluent (2×)	Concentrated buffered diluent used to dilute standard and samples.	1 vial
Streptavidin-HRP A (200×)	200× Streptavidin conjugated to horseradish peroxidase.	1 vial
Reagent Diluent (10×)	A 10× concentrated buffered protein base used to dilute detection antibody and HRP.	1 vial
Assay Diluent	A buffered protein base with preservatives.	1 vial
Normal goat serum	Diluted, heat-inactive normal goat serum	1 vial
Wash Buffer Concentrate (25×)	A 25× concentrated solution of buffered surfactant with preservatives.	1 vial
Color Reagent A	Stabilized hydrogen peroxide.	1 vial
Color Reagent B	Stabilized chromogen (tetramethylbenzidine).	1 vial
Stop Solution	2 N sulfuric acid.	1 vial
Plate Sealers	Adhesive strips.	3 strips

B. STORAGE

<table>
<tr><td>Unopened Kit</td><td colspan="2">Store at 2-8 °C. Do not use past kit expiration date.</td></tr>
<tr><td rowspan="10">Opened/ Reconstituted Reagents</td><td>Streptavidin-HRP A</td><td rowspan="6">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Diluted Wash Solution</td></tr>
<tr><td>Unmixed Color Reagent A</td></tr>
<tr><td>Unmixed Color Reagent B</td></tr>
<tr><td>Stop Solution</td></tr>
<tr><td>Assay Diluent</td></tr>
<tr><td>Normal goat serum</td><td rowspan="2">Prepare fresh for each assay.</td></tr>
<tr><td>Standard</td></tr>
<tr><td>Detection antibody</td><td>Aliquot and store for up to 1 month at -20 °C in a manual defrost freezer. *</td></tr>
<tr><td>Reagent Diluent(10×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Reagent Diluent(1×). Prepare fresh for each assay.</td></tr>
<tr><td>Calibrator Diluent(2×)</td><td>May be stored for up to 1 month at 2-8 °C.*
Use and discard diluted Calibrator Diluent (1×). Prepare fresh for each assay.</td></tr>
<tr><td>Microplate Wells</td><td>Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*</td></tr>
</table>

* Provided this is within the expiration date of the kit.

C. OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Test tubes for dilution of standards.
- 100mL and 500 mL graduated cylinder.
- Horizontal orbital microplate shaker capable of maintaining a speed of 500±50 rpm.

D. PRECAUTION

- Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.
- Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.
- The Stop Solution provided with this kit is an acid solution.
- Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

VI. PREPARATION

A. SAMPLE COLLECTION AND STORAGE

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 ° C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 ° C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

B. SAMPLE PREPARATION

Serum samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent (1×).

C. REAGENT PREPARATION

Note: *Bring all reagents to room temperature before use.*

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of Wash Buffer Concentrate (25×) into deionized or distilled water to prepare 500 mL of Wash Buffer.

Reagent Diluent (1×) - Add 5 mL of Reagent Diluent (10×) into 45 mL of deionized or distilled water to prepare 50 mL of Reagent Diluent (1×).

Calibrator Diluent (1×) - Use deionized or distilled water to prepare Calibrator Diluent (1×).

Detection Antibody- Reconstitution Volume refer to vial label with Reagent Diluent (1×). Aliquot and store if needed. Dilute stock solution in Reagent Diluent (1×) with 2% heat inactivated normal goat serum (NGS) (200 uL NGS per 10 mL solution) to the working concentration of 50 ng/mL. Prepare at least 15 minutes prior to use.

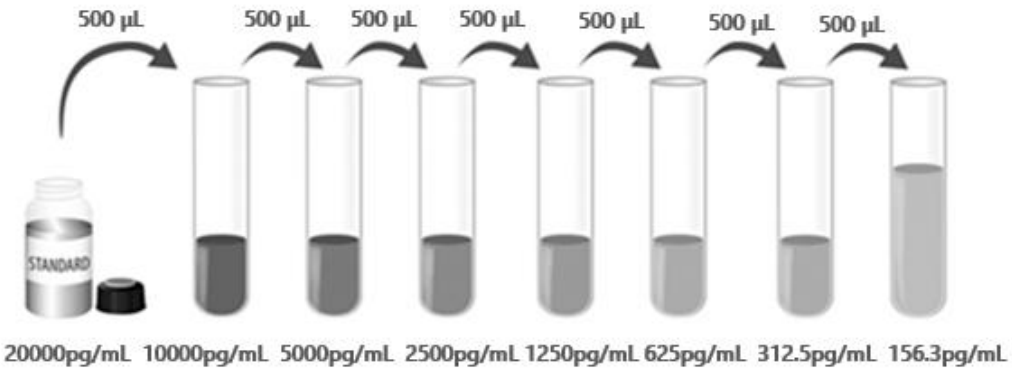
Streptavidin-HRP A (1×) **-** Dilute to the working concentration specified on the vial label using Reagent Diluent (1×).

Substrate Solution - Color Reagent A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

PD-L1/B7-H1 Standard – Refer to the vial label for the reconstitution volume* using Calibrator Diluent. This reconstitution produces a stock solution of 20000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

*if you have any question, please seek help from our Technical Support.

Pipette 500 µL of Calibrator Diluent 1× into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 10000 pg/mL standard serves as the high standard. The **Calibrator Diluent 1×** serves as the zero standard (0 pg/mL).



D. TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- It is recommended that the samples be pipetted within 15 minutes.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

VII. ASSAY PROCEDURE

Note: *Bring all reagents and samples to room temperature before use. It is recommended that all samples and standards be assayed in duplicate.*

1. Prepare all reagents and working standards as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent to each well.
4. Add 100 µL of Standard, or prepared sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker set at 500rpm. A plate layout is provided for a record of standards and samples assayed. (Serum samples may require dilution. See Sample Preparation section.)
5. Aspirate each well and wash, repeating the process two or three times. Wash by filling each well with Wash Solution (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of the Detection Antibody diluted in Reagent Diluent with NGS, to each well. Cover with a new adhesive strip and incubate 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of the working dilution of Streptavidin-HRP A to each well. Cover the plate and incubate for 30 minutes at room temperature. Avoid placing the plate in direct light.
9. Repeat the aspiration/wash as in step 5.
10. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. Avoid placing the plate in direct light.
11. Add 50 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
12. Determine the optical density of each well immediately, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.
13. **CALCULATION OF RESULTS**

    Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the PD-L1/B7-H1 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

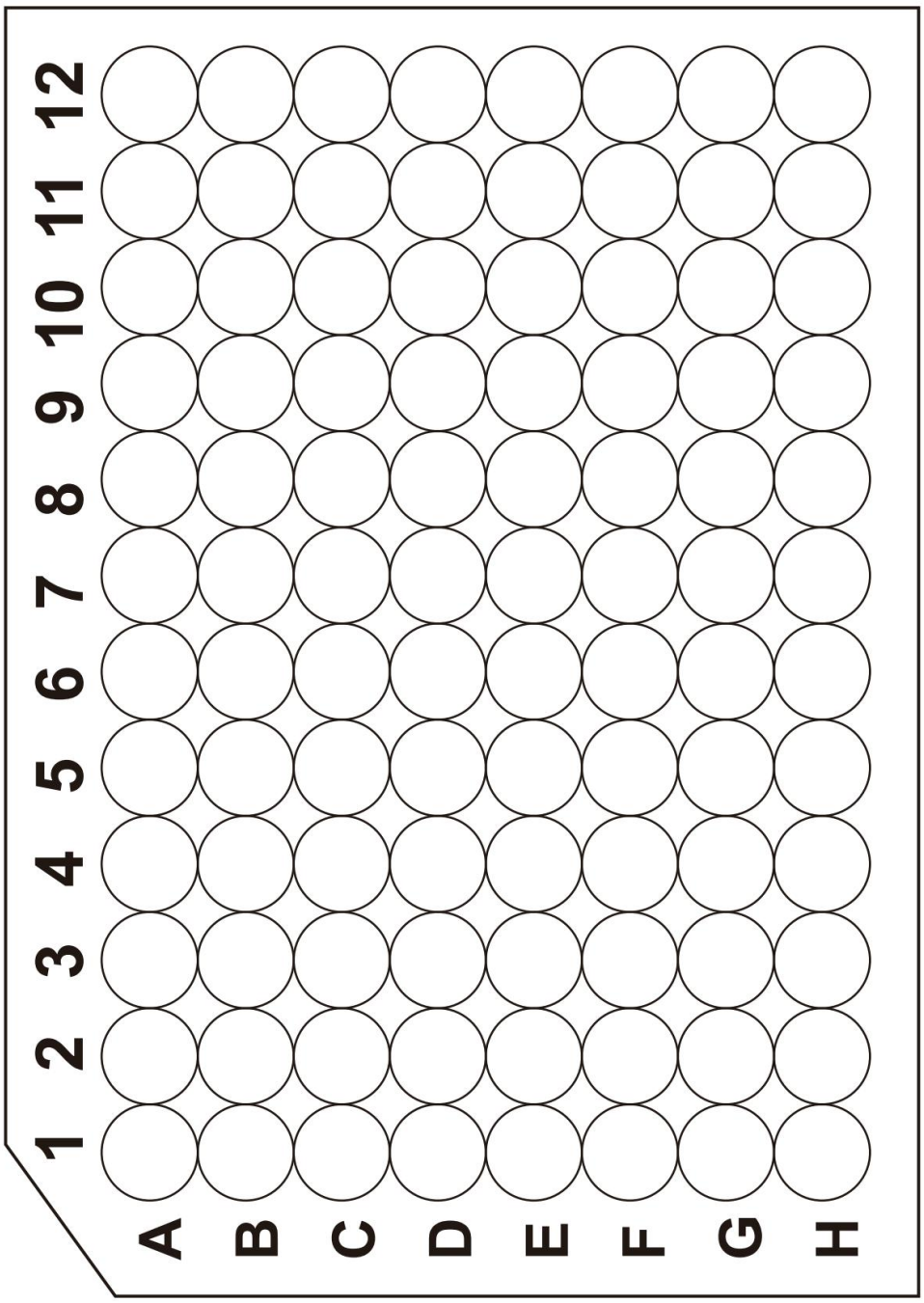
If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

VIII. REFERENCES

1. Ceeraz, S. et al. (2013) Trends Immunol. 34:556.
2. Tamura, H. et al. (2001) Blood 97:1809.
3. Chen, L. et al. (2007) J. Immunol. 178:6634.
4. Kuang, D.-M. et al. (2014) J. Clin. Invest. 124:4657.
5. Tsushima, F. et al. (2007) Blood 110:180.
6. Mazanet, M.M. and C.C.W. Hughes (2002) J. Immunol. 169:3581.
7. Cao, Y. et al. (2010) Cancer Res. 71:1235.
8. Scandiuzzi, L. et al. (2014) Cell Rep. 6:625.
9. Dong, H. et al. (2002) Nat. Med. 8:793.
10. Azuma, T. et al. (2008) Blood 111:3635.
11. Butte, M.J. et al. (2008) Mol. Immunol. 45:3567.
12. Park, J.-J. et al. (2010) Blood 116:1291.
13. Ritprajak, P. et al. (2010) J. Immunol. 184:4918.
14. Herold, M. et al. (2015) J. Immunol. 195:3584.
15. Zhang, J. et al. (2015) Thorac. Cancer 6:534.
16. Zheng, Z.X. et al. (2014) Chin. J. Cancer Res. 26:104.
17. Cheng, S. et al. (2015) Int. J. Biol. Markers 30:e364.
18. Zeng, Z. et al. (2011) PLoS One 6:e23621.
19. Frigola, X. et al. (2011) Clin. Cancer Res. 17:1915.
20. Baral, A. et al. (2014) Oncol. Lett. 8:1195.
21. Chen, Y. et al. (2011) Cytokine 56:231.
22. Frigola, X. et al. (2012) Immunol. Lett. 142:78.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



产品信息及操作手册

人 PD-L1/B7-H1 Valukine™ ELISA 试剂盒

目录号：VAL117

适用于定量检测天然和重组人 PD-L1/B7-H1 的浓度

科研专用，不可用于临床诊断

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目录

I. 背景

程序性死亡因子配体 1(PD-L1)，也被称为 B7- H1 和 CD274，是一种大约 65 kDa 的跨膜糖蛋白，属于 B7 家族的免疫调节分子(1)。PD-L1 表达于被炎症激活的免疫细胞，包括巨噬细胞、T 细胞和 B 细胞 (2-5)，角质形成细胞 (6, 7)，内皮细胞和肠道上皮细胞 (6, 8)，以及各种癌细胞和黑色素瘤 (9, 10)。PD-L1 能与 PD-1 和 T 细胞 B7-1/CD80 结合 (5, 6, 10-13)，抑制 T 细胞的活化和增殖 (3, 6, 12, 14)，诱导活化的 T 细胞凋亡 (9)。它通过促进 T 细胞失活 (5, 12)和调节 T 细胞发育 (14)，在免疫耐受过程中发挥作用。PD-L1 有利于抗炎过程中的 IL-10 和 IL-22 生成树突状细胞的发育 (3, 8)，抑制 Th17 细胞的发育(14)。在癌症中，PD-L1 能够抵抗 T 细胞介导的裂解，增强上皮细胞向间质转化的能力，增强 Th22 细胞的致瘤功能 (4, 7, 10, 13)。在癌症患者的血浆和胶质瘤患者的脑脊液中，其可溶性 PD-L1 会有所升高 (15-20)。它可以被成熟的树突状细胞释放，并保留结合 PD-1 和诱导 T 细胞凋亡的能力 (19, 21, 22)。

II. 概述

A. 检测原理

本实验采用双抗体夹心 ELISA 法。抗人 PD-L1/B7-H1 捕获抗体包被于微孔板上，经过孵育，样品和标准品中的 PD-L1/B7-H1 会与固定在板上的抗体结合，游离的成分被洗去；接着加入生物素化的抗人 PD-L1/B7-H1 检测抗体进行孵育，洗涤去除未结合的物质后，加入链霉亲和素标记的辣根过氧化物酶(streptavidin-HRP)孵育。洗涤后，加入显色底物，避光显色。溶液颜色与结合的目标蛋白成正比；加入终止液；用酶标仪测定吸光度。

B. 检测局限

- 仅供科研使用，不可用于体外诊断；
- 该试剂盒适用于细胞培养上清、人血清和血浆样本；
- 请在试剂盒有效期内使用；
- 不同试剂盒及不同批号试剂盒的组分不能混用；
- 样本值若大于标准曲线的最高值，应将样本用标准品稀释剂（1×）稀释后重新检测；
- 检测结果的不同可由多种因素引起，包括实验人员的操作、移液器的使用方式、洗板技术、反应时间或温度、试剂盒的储存等。

III. 优势

A. 精确度

板内精确度（同一板内不同孔间的精确度）

已知浓度的三个样本，在同一板内分别检测 20 次，以确定板内精确度。

板间精确度（不同板之间的精确度）

已知浓度的三个样本，在不同板中分别检测 20 次，以确定板间精确度。

	板内精确度			板间精确度		
样本	1	2	3	1	2	3
平均值 (pg/mL)	590.7	1596.9	5107.3	596.8	1663.3	5850.1
标准差	18.2	95.1	409.8	18.5	64.7	555.5
CV%	3.1	6.0	8.0	3.1	3.9	9.5

B. 回收率

在不同类型样本中掺入检测范围内不同水平的人PD-L1/B7-H1，测定其回收率。

样本类型	平均回收率	范围
细胞培养上清 (n=4)	97	83 - 107%
血清 (n=5)	117	114 - 119%
血浆 (n=4)	96	85 - 109%

C. 灵敏度

人 PD-L1/B7-H1 的最低可测剂量（MDD）一般小于 11.5pg/mL。

MDD 是根据 20 个重复的零标准品孔的吸光度值的平均值加两倍标准差计算得到的相对应浓度。

D. 校正

此 ELISA 试剂盒经由 R&D Systems®生产的 NS0 表达的高纯度重组人 PD-L1/B7-H1 蛋白所校正。

E. 线性

在不同类型样本中掺入高浓度的人PD-L1/B7-H1，然后用稀释剂将样本稀释到检测范围内，测定其线性。

稀释倍数		细胞培养上清（n=4)	血清（n=5)	血浆（n=4)
1:2	平均值/期待值（%）	92	106	100
	范围（%)	91 - 93	97 - 113	83 - 110
1:4	平均值/期待值（%）	94	107	96
	范围（%)	92 - 96	100 - 115	85 - 112
1:8	平均值/期待值（%）	95	110	98
	范围（%)	93 - 98	105 - 118	81 - 117
1:16	平均值/期待值（%）	100	114	101
	范围（%)	95 - 105	107 - 123	88 - 124

F. 样本值

血清样本 -使用本试剂盒检测了 5 份人血清样本中 PD-L1/B7-H1 的水平。5 份样本的检测值在 392-562 pg/mL 之间，平均值为 486.2 pg/mL。

血浆样本 - 使用本试剂盒检测了4份人血浆样本中PD-L1/B7-H1的水平。样本的检测值均低于最低标准品，156.3 pg/mL。

G. 特异性

此 ELISA 法可检测天然及重组人 PD-L1/B7-H1 蛋白。对制备的 100ng/mL 的下列因素进行了测定，无交叉反应或干扰。

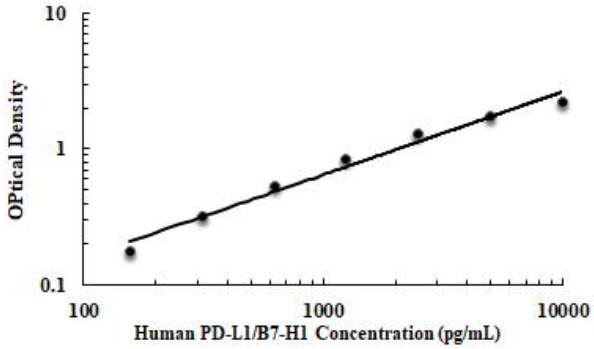
重组人蛋白	其他重组蛋白
B7-1/Fc Chimera	小鼠B7-H1 Fc Chimera
B7-2 Fc Chimera	大鼠 B7-1 Fc Chimera
B7-H2 Fc Chimera	
B7-H3	
B7-H4	
B7-H6 Fc Chimera	

重组人 PD-1/Fc Chimera 没有交叉反应，但当浓度> 50 ng/mL 时有干扰。

IV. 实验

标准曲线实例

该标准曲线数据仅供参考，每次实验应绘制其对应的标准曲线。



pg/mL	OD	Average	Corrected
0	0.075 0.067	0.071	-
156.25	0.250 0.248	0.249	0.178
312.5	0.385 0.394	0.390	0.319
625	0.596 0.604	0.600	0.529
1250	0.906 0.899	0.903	0.832
2500	1.354 1.357	1.356	1.285
5000	1.806 1.809	1.808	1.737
10000	2.317 2.284	2.301	2.230

V. 试剂盒组成及储存

A. 试剂盒组成

组成	描述	规格
人PD-L1/B7-H1Microplate	包被小鼠抗人PD-L1/B7-H1抗体的96孔聚苯乙烯板，8孔×12条	1块板
人PD-L1/B7-H1 Standard	标准品（冻干粉），参考瓶标签进行重溶	2瓶
人PD-L1/B7-H1 Detection antibody	生物素化的人PD-L1/B7-H1检测抗体，冻干粉，参考瓶标签进行重溶	1瓶
Streptavidin-HRP A (200×)	200×浓缩的链霉亲和素标记的HRP	1瓶
Reagent Diluent (10×)	浓缩的试剂稀释液（10×）	1瓶
Calibrator Diluent (2×)	浓缩的样品和标准品稀释剂（2×）	1瓶
Assay Diluent	检测液	1瓶
Normal goat serum	稀释的热灭活正常山羊血清	1瓶
Wash Buffer Concentrate (25×)	浓缩洗涤缓冲液（25×）	1瓶
Color Reagent A	显色液A	1瓶
Color Reagent B	显色液B	1瓶
Stop Solution	终止液	1瓶
Plate Sealers	封板膜	3张

B. 试剂盒储存

<table>
<tr><td>未开封试剂盒</td><td colspan="2">2-8℃储存；请在试剂盒有效期内使用</td></tr>
<tr><td rowspan="10">已打开，稀释或重溶的试剂</td><td>链霉亲和素-HRP A</td><td rowspan="7">2-8℃储存，最多 30 天*</td></tr>
<tr><td>洗涤缓冲液（1×）</td></tr>
<tr><td>显色剂 A</td></tr>
<tr><td>显色剂 B</td></tr>
<tr><td>检测液</td></tr>
<tr><td>山羊血清</td></tr>
<tr><td>终止液</td></tr>
<tr><td>标准品</td><td>使用时新鲜配制*</td></tr>
<tr><td>检测抗体</td><td>分装，-20℃储存，最多 30 天*</td></tr>
<tr><td>标准品稀释剂（2×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×稀释液</td></tr>
<tr><td>试剂稀释液（10×）</td><td>2-8℃储存，最多 30 天*
请每次使用新鲜配制的 1×稀释液</td></tr>
<tr><td>包被的微孔板条</td><td>将未用的板条放回带有干燥剂的铝箔袋内，密封：2-8℃储存，最多 30 天*</td></tr>
</table>

*必须在试剂盒有效期内

C. 实验所需自备试验器材

- 酶标仪（可测量 450nm 检测波长的吸收值及 540nm 或 570nm 校正波长的吸收值）
- 高精度加液器及一次性吸头
- 蒸馏水或去离子水
- 洗瓶（喷瓶）、多通道洗板器或自动洗板机
- 用来稀释标准品的试管
- 100mL 及 500mL 量筒
- 振荡器（200-1500rpm）

D. 注意事项

- 试剂盒中的终止液是酸性溶液，使用时请做好眼镜、手、面部及衣服的防护。
- 试剂盒中的一些组分，可能引起皮肤、眼睛和呼吸道刺激或皮肤过敏反应。避免吸入。
- 实验穿戴防护衣服、手套、眼睛和脸的保护罩。使用后请彻底洗手。

VI. 实验前准备

A. 样品收集及储存

细胞培养上清液：颗粒物应离心去除；立刻检测样本。样本收集后若不及时检测，需按一次使用量分装，冻存于-20℃冰箱内，避免反复冻融。

血清样本: 用血清分离管（SST）分离血清。使血样室温凝集 2 小时或 4℃过夜，然后 1000 x g 离心 15 分钟。吸取血清样本之后即刻用于检测，或者分装，-20℃贮存备用。避免反复冻融。

血浆样本: 使用 EDTA、肝素钠或枸橼酸钠作为抗凝剂收集血浆。然后 1000 x g 离心 15 分钟，需在 30 分钟内收集血浆样本之后即刻用于检测，或者分装，-20℃贮存备用。避免反复冻融。

B. 样本准备工作

血清样本需要用标准品稀释剂（1×）2倍稀释后进行检测，即100μL血清+100μL标准品稀释剂（1×）。

C. 检测前准备工作

使用前请将所有试剂放置于室温

洗涤液：从冰箱中取出的浓缩洗涤液可能有结晶，属于正常现象；放置室温，轻摇混匀，待结晶完全溶解后再配制洗涤液。可将 20mL 浓缩洗涤液用蒸馏水或去离子水稀释配置成 500mL 工作浓度的洗涤液。可 4℃存储。

试剂稀释液（1×）：加 5mL 试剂稀释液（10×）至 45mL 蒸馏水或去离子水中，制成 50mL 试剂稀释液（1×）。

标准品稀释剂（1×）：使用蒸馏水或去离子水稀释配置成标准品稀释剂（1×）。

检测抗体： 参考检测抗体瓶标签指示，用试剂稀释液（1×）将冻干粉进行重溶。再用添加了 2%热灭活的正常山羊血清（NGS）（每 10 毫升溶液加 200μL NGS）的试剂稀释液（1×）稀释至工作浓度 50ng/mL，至少在使用前 15 分钟准备。

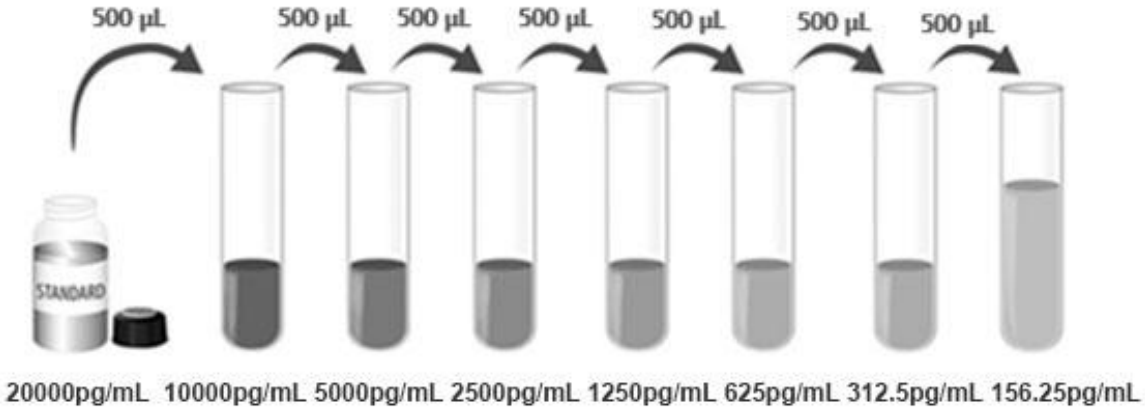
链霉亲和素- HRP A：用试剂稀释液（1×）将链霉亲和素- HRP A（200×）稀释至工作浓度链霉亲和素- HRP A（1×）。

显色剂：按试验所需用量（100μL/孔）将显色液 A 和显色液 B 等体积混合，避光保存，现用现配，须在 15 分钟内使用。

标准品：参照冻干标准品瓶身注明的方式重溶冻干标准品*，得到浓度为 20000pg/mL 标准品母液。轻微震摇至少 15 分钟，使其充分溶解。

***如有疑问，请咨询我们的技术支持。**

每个稀释管中加入 500μL 标准品稀释剂（1×）。将标准品母液参照下图做系列稀释，每管须充分混匀后再移液到下一管。10000pg/mL 管作标准曲线最高点，标准品稀释剂（1×）可用作标准品零点（0pg/mL）。



D. 技术小提示

- 当混合或重溶蛋白液时，尽量避免起沫；
- 为了避免交叉污染，配置不同浓度标准品、上样、加不同试剂都需要更换枪头。另外不同试剂请分别使用不同的移液槽；
- 建议 15 分钟内完成一块板的上样；
- 每次孵育时，正确使用封板膜可保证结果的准确性；
- 混合后的显色剂在上板前应为无色，请避光保存；加入微孔板后，将由无色变成不同深度的蓝色；
- 终止液上板顺序应同显色底物上板顺序一致；加入终止液后，孔内颜色由蓝变黄；若孔内有绿色，则表明孔内液体未混匀，请充分混合。

VII. 操作步骤

使用前请将所有试剂和样本放置于室温，建议所有的实验样本和标准品做复孔检测。

1. 按照上一节的说明，准备好所有需要的试剂和标准品；
2. 从已平衡至室温的密封袋中取出微孔板，未用的板条请放回铝箔袋内，重新封口；
3. 在每个微孔内加入 50μL 检测液；
4. 分别将不同浓度标准品，实验样本或者质控品加入相应孔中，每孔 100μL。用封板膜封住反应孔，置于振荡器上，500rpm，室温孵育 2 小时。说明书提供了一张 96 孔模板图，可用于记录标准品和试验样本的板内位置；（血清样本需要稀释，详情参见样本制备部分。）
5. 将板内液体吸去，使用洗瓶、多通道洗板器或自动洗板机洗板。每孔加洗涤液 400μL，然后将板内洗涤液吸去。重复操作 2-3 次。每次洗板尽量吸去残留液体会有助于得到好的实验结果。最后一次洗板结束，请将板内所有液体吸干或将板倒置，在吸水纸拍干所有残留液体；
6. 在每个微孔内加入 100μL 配置好的检测抗体。用封板膜封住反应孔，室温孵育 2 小时；
7. 重复第 5 步洗板操作；
8. 在每个微孔内加入 100μL 稀释好的链霉亲和素- HRP A 工作液。用封板膜封住反应孔，室温孵育 30 分钟，**注意避光；**
9. 重复第 5 步洗板操作；
10. 在每个微孔内加入 100μL 显色剂，室温孵育 30 分钟，**注意避光；**
11. 在每个微孔内加入 50μL 终止液，请轻拍微孔板，使溶液混合均匀。孔内溶液颜色会从蓝色变为黄色；
12. 加入终止液后 30 分钟内，使用酶标仪测量 450nm 的吸光度值，设定 540nm 或 570nm 作为校正波长。如果没有使用双波长校正，结果准确度可能会受影响；
13. **计算结果：**将每个标准品和样品的校正吸光度值（OD450-OD540/OD570），复孔读数取平均值，然后减去平均零标准品 OD 值。使用计算机软件作四参数逻辑（4-PL）曲线拟合创建标准曲线。另一种方法是，可以通过绘制标准品浓度做对数与相应 OD 值对数生成曲线，并通过回归分析确定最佳拟合线。通过样本的 OD 值，可从标准曲线上得到样本中人 PD-L1/B7-H1 的浓度。如果样品被稀释，从标准曲线读取的浓度必须乘以稀释倍数。

VIII. 参考文献

1. Ceeraz, S. et al. (2013) Trends Immunol. 34:556.
2. Tamura, H. et al. (2001) Blood 97:1809.
3. Chen, L. et al. (2007) J. Immunol. 178:6634.
4. Kuang, D.-M. et al. (2014) J. Clin. Invest. 124:4657.
5. Tsushima, F. et al. (2007) Blood 110:180.
6. Mazanet, M.M. and C.C.W. Hughes (2002) J. Immunol. 169:3581.
7. Cao, Y. et al. (2010) Cancer Res. 71:1235.
8. Scandiuzzi, L. et al. (2014) Cell Rep. 6:625.
9. Dong, H. et al. (2002) Nat. Med. 8:793.
10. Azuma, T. et al. (2008) Blood 111:3635.
11. Butte, M.J. et al. (2008) Mol. Immunol. 45:3567.
12. Park, J.-J. et al. (2010) Blood 116:1291.
13. Ritprajak, P. et al. (2010) J. Immunol. 184:4918.
14. Herold, M. et al. (2015) J. Immunol. 195:3584.
15. Zhang, J. et al. (2015) Thorac. Cancer 6:534.
16. Zheng, Z.X. et al. (2014) Chin. J. Cancer Res. 26:104.
17. Cheng, S. et al. (2015) Int. J. Biol. Markers 30:e364.
18. Zeng, Z. et al. (2011) PLoS One 6:e23621.
19. Frigola, X. et al. (2011) Clin. Cancer Res. 17:1915.
20. Baral, A. et al. (2014) Oncol. Lett. 8:1195.
21. Chen, Y. et al. (2011) Cytokine 56:231.
22. Frigola, X. et al. (2012) Immunol. Lett. 142:78.

96 孔模板图

请使用 96 孔模板图来记录标准品及样本在板内的位置