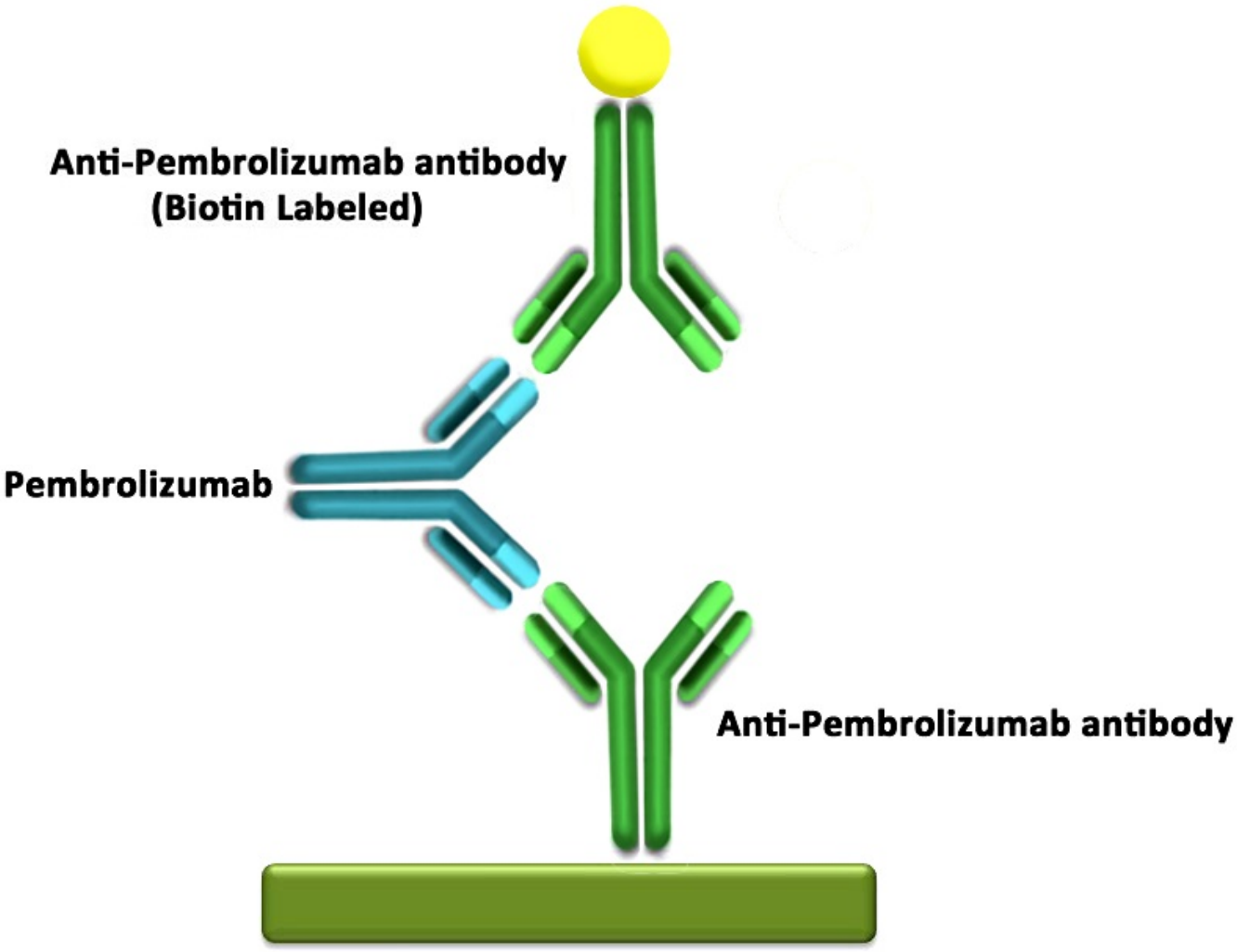
Pembrolizumab ELISA Detection Kit

Cat. No. L00696 Version 06152017



The operator should read technical manual carefully before using this product.

Research use only. Not for diagnostic use.

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I. DESCRIPTION

Pembrolizumab is a humanized antibody used in cancer immunotherapy that targets the programmed cell death 1 (PD-1) receptor. It blocks a protective mechanism on cancer cells, and allows the immune system to destroy those cancer cells. The drug was initially used to treat metastatic melanoma.

Pembrolizumab ELISA Detection Kit is a sandwich enzyme-linked immunoassay which can be used for quantitative detection of Pembrolizumab drug in samples. The Capture Plate is pre-coated with an Anti-Pembrolizumab Monoclonal Antibody. When free Pembrolizumab or specimen is added to the well, the Pembrolizumab will be captured by the plate. Other unbound molecules are removed by the wash step. Then the Biotin-labeled detection antibody and Horseradish peroxidase conjugated streptavidin are added to react with the TMB substrate to develop a blue product in the solution. The reaction is stopped by adding stop solution and the color turns yellow which can be read at 450 nm by a Microtiter plate reader. The color developed is proportional to the amount of Pembrolizumab present in the testing samples. In optimized test conditions, each absorbance value is indicated to the individual Pembrolizumab amount in samples. The

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Pembrolizumab standards of known concentration and corresponding absorbance values are used to form a standard curve. With the standard curve, Pembrolizumab amount present in the unknown sample is calculated by transforming its absorbance value.

II. KEY FEATURES

Feature	Specification
Sensitivity	0.295ng/ml
Detection Range	1.5625-100ng/ml
Precision	Inter-assay: 6.51%.; Intra-assay: 3.50%.
Recovery	Recovery range of this kit is between 85%-115%.
Test Samples	Human serum/plasma ,mouse serum/plasma
Conveniency	All reagents and buffers for test are provided complete the test within 2 hours

III. KIT CONTENTS

Reagents and buffers for Pembrolizumab detection.

Component	Quantity	Part No.
Pembrolizumab Capture Plate	1 plate (8 wells x 12 strips)	696-80
Detection Antibody	12 mL	696-20
Streptavidin-HRP	12 mL	696-30
Pembrolizumab Standard Stock (10μg/mL)	50 μL	696-10
Sample Dilution Buffer	60 mL	696-60
20 × Wash Solution	40 mL	696-70
TMB Substrate	12 mL	696-40
Stop Solution	6 mL	696-50
Plate Sealer	2 pieces	N/A
User Manual	1 copy	N/A

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IV. STORAGE

The unopened kit is stable for at least 12 months if stored at 2-8 °C, and the opened kit is stable for up to 1 month at 2-8 °C.

V. REAGENTS/EQUIPMENT (NOT SUPPLIED)

Microtiter plate reader capable of measuring absorbance at 450 nm
Automated microplate washer to wash the plate
Deionized or distilled water to dilute 20 x Wash Solution
Graduated cylinder to prepare Wash Solution
Plastic container to store Wash Solution
Tubes to aliquot and dilute samples
Precision pipettes to deliver 10μL, 100μL, 200μL and 1000μL content
10μL, 100μL, 200μL and 1000μL pipette tips
Multichannel pipettor
Disposable reagent reservoir
Paper towel
Laboratory timer
Refrigerator to store samples and kit components

VI. PROTOCOL

- All reagents in the kit and test samples should be equilibrated to room temperature before use.
- Preliminary experiments should be performed to optimize the sample dilution.
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Reagent Preparation

- If any precipitate is found in the 20 × Wash Solution, incubate the bottle in water bath (up to 50 °C) with occasional mixing until all the precipitate is dissolved.

1 x Wash Solution: Dilute *20 × Wash Solution* by 1:19 v/v with deionized or distilled water. For example, dilute 40 mL of *20 × Wash Solution* with 760 mL of deionized or distilled water to make 800 mL of 1 × *Wash Solution*. Store at 2-8 °C.

Pembrolizumab Standards Preparation

- The kit provides *Pembrolizumab standard stock* for sample test.
- All reagents in the kit and test samples should be equilibrated to room temperature before use.

1. Label nine 1.5 mL Eppendorf tubes with “100 ng/mL”, “50ng/mL”, “25ng/mL”, “12.5ng/mL”, “6.25ng/mL”, “3.125ng/mL”, “1. 5625ng/mL” and “0ng/mL”.
2. Pipette 10μL of *Pembrolizumab Standard stock* and 990μL of *Sample Buffer* into the tube labeled with “100 ng/mL” and vortex it.

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3. Pipette 500μLof *Sample Buffer* into the rest of the empty tubes.
4. Pipette 500μLof 100ng/mL of Pembrolizumab standard solution to the tube labeled with “50ng/mL” and vortex it to make the standard be 50ng/mL.
5. Similarly, prepare the rest of the standard series (25，12.5，6.25，3.125，1.5625ng/mL).

Samples preparation

Handle serum or plasma samples in accordance with NCCLS (National Committee for Clinical Laboratory Standards) guidelines for preventing transmission of blood-bore infection.

Human Serum: Use a blood separator tube and allow sample to clot for 30 minutes, then centrifuge for 10 minutes at 1000 x g. Run assay immediately, otherwise aliquot and store sample below -20°C. Avoid repeat thaw freeze cycle. When the human serum is tested, it should be diluted higher than 1000-fold.

Human Plasma: Treat blood with citrate, EDTA or heparin as anticoagulant. Centrifuge for 10 minutes at 1000 x g within 30 minutes for plasma collection. Then Run assay immediately. Otherwise aliquot and store sample below -20°C. Avoid repeat thaw-freeze cycle. When the human plasma is tested, it should be diluted higher than 1000-fold.

Mouse and Rat serum/plasma: Use a blood separator tube, then centrifuge for 10 minutes at 1000 x g. Run assay immediately, otherwise aliquot and store sample below -20°C. Avoid repeat thaw freeze cycle. When the mouse and Rat serum /plasma is tested, it should be diluted higher than 20 fold.

Perform preliminary experiment to determine the optimum detection sample dilution.

The standard curve and sample dilution design in the following table.

	Standard Curve (ng/mL)		Sample dilution									
	Duplicate 1	**Duplicate 2**	Sample 1	Sample 1	5	6	7	8	9	10	11	12
A	100	100	Non-diluted	Non-diluted								
B	50	50	1/10	1/10								
C	25	25	1/100	1/100								
D	12.5	12.5	1/1000	1/1000								
E	6.25	6.25	1/10000	1/10000								
F	3.125	3.125										
G	1.5625	1.5625										
H	0	0										

Capture Plate Preparation

- It is recommended that all Pembrolizumab standards and samples be prepared in duplicate.
- Count the strips for the assay and make sure the strips are tightly snapped in the plate frame.
- Leave the unused strips in the foil pouch and store at 2-8 °C. The strips must be stored in the closed foil pouch to prevent moisture because the moisture can damage the Capture Plate.

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Test Procedure

Pembrolizumab standard and samples Incubation

1. Add 100μL of Pembrolizumab standard solution and samples to the corresponding wells.
2. Cover the plate with *Plate Sealer* and incubate at 37 °C for 60min.
3. Remove the *Plate Sealer a*nd wash the plate with 260 μL of *1 x Wash Solution* for four times.
4. Pat the plate on paper towel to remove residual liquid in the wells after wash step.

Detection Antibody Incubation

1. Add 100μL of *Detection Antibody* to all the wells.
2. Cover the plate with *Plate Sealer* and incubate at 37 °C for 30min.
3. Remove the Plate Sealer and wash the plate with 260 μL of *1 x Wash Solution* for four times.
4. Pat the plate on paper towel to remove residual liquid in the wells after wash step.

Streptavidin-HRP Incubation

1. Add 100μL of *Streptavidin-HRP* to all the wells.
2. Cover the plate with *Plate Sealer* and incubate at 37 °C for 10min.
3. Remove the *Plate Sealer* and wash the plate with 260 μL of *1 x Wash Solution* four times.
4. Pat the plate on paper towel to remove residual liquid in the wells after wash step.

Substrate Reaction and Absorbance Measurement

1. Add 100 μL of TMB Substrate to all the wells and incubate at 25 °C for 15-20 minutes (start timing from when the TMB Substrate was added to the first well) and protect it from light.
2. Add 50 μL of *Stop Solution* to all the wells to stop the enzyme reaction.
3. Read the plate on Microtiter plate reader at 450 nm.

Note: The substrate reaction time is determined by the temperature, the perfect reaction temperature is 25℃.When the temperature is below 25℃, appropriate extend the reaction time

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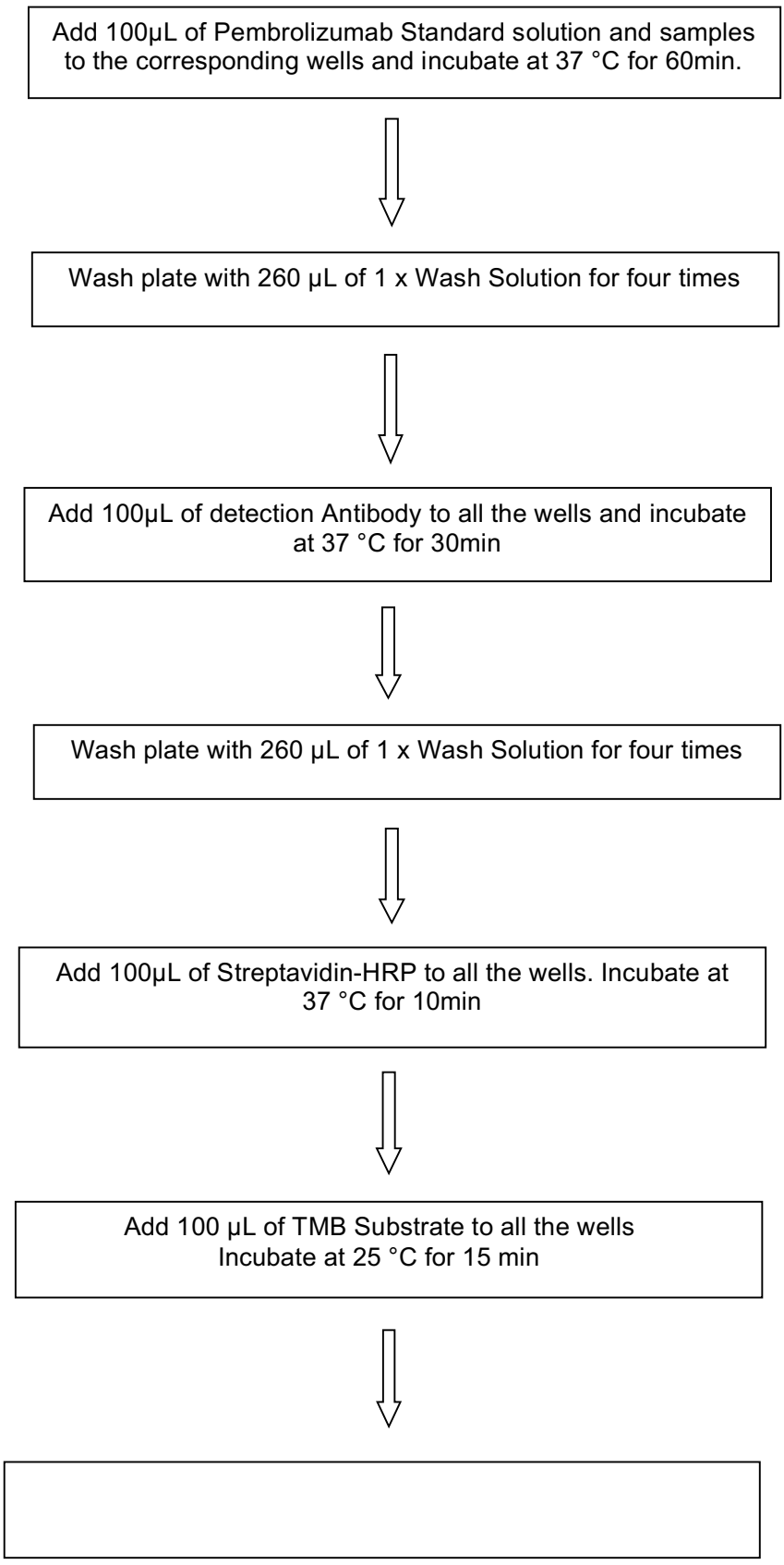
VII. ASSAY PROCEDUR SUMMARY



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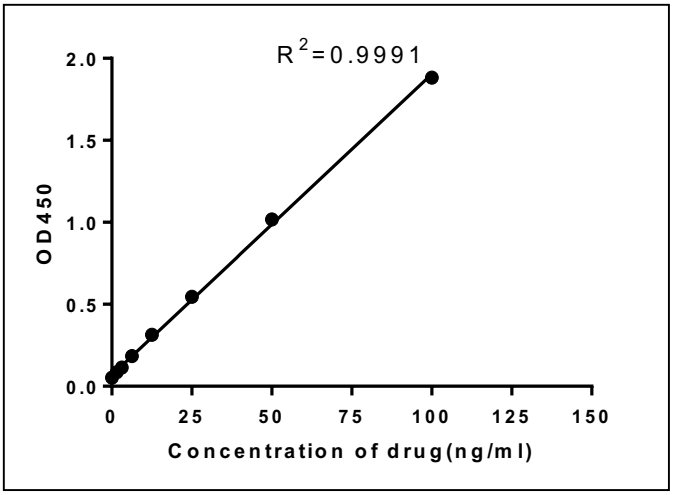
VIII. TYPICAL ASSAY DATA

The standard curve below was provided for demonstration only. Operator should set up standard curve to precisely determine Pembrolizumab drug each time.



Pembrolizumab Standard	OD_{450}		
(ng/mL)	Duplicate 1	Duplicate 2	Average
100	1.915	1.850	1.883
50	1.025	1.008	1.017
25	0.541	0.550	0.546
12.5	0.307	0.321	0.314
6.25	0.185	0.183	0.184
3.125	0.112	0.115	0.114
1.5625	0.081	0.090	0.086
0	0.053	0.052	0.053

IX. PRECISION

Intra-assay: Three different known levels of control were spiked into sample buffer as test samples. All samples were tested 10 times on the same plate to evaluate intra-assay precision of the kit. Intra-assay precision of this kit is 3.50%.

Inter-assay: Three different known levels of control were spiked into sample buffer as test samples. All samples were tested in 6 separate assays to evaluate intra-assay precision of the kit. Inter-assay precision of this kit is 6.51%.

X. SENSITIVITY

The minimum detectable dose (MDD) of the assay is between 0.086-0.561 ng/mL. The mean MDD is 0.295ng/mL.

XI. RECOVERY

Recovery range of this kit is between 85%-115%.

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XII. TROUBLESHOOTING

Problem	Probable Cause	Solution
Poor Precision	Wells are not washed or aspirated properly	Make sure the wash apparatus works properly and wells are dry after aspiration
	Wells are scratched with pipette tip or washing needles	Dispense and aspirate solution into and out of wells with caution
	Particulates are found in the samples	Remove any particulates by centrifugation prior to the assay
Poor Standard Curve	Improper preparation of standards	Prepare new standards as the manual describes
	Wells are not washed or aspirated properly	Make sure the wash apparatus works properly and wells are dry after aspiration
	Pipetting error	Check pipette calibration and repeat assay
	Components are used from other lots or sources	Never substitute any components from another kit
	Components are not brought to room temperature prior to assay	Repeat assay with components that have been equilibrated to room temperature
	Incubation steps are performed at wrong temperatures	Perform incubation step as the manual describes
Weak/No Signal	TMB substrate are not added or added at the wrong time	Follow the manual to add the substrate properly
	Components are used from other lots or sources	Use only lot-specific components
	TMB substrate is contaminated	Use new TMB substrate
	Did not add the proper volumes of reagents	Repeat assay with the required volumes in manual
	Did not incubate the plate for proper time or temperature	Follow the manual to repeat assay
	Did not read the plate immediately after stop solution was added	Read the plate within 30 minutes after adding stop solution

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High Background	Plate is not washed properly	Make sure the wash apparatus works properly
	TMB substrate is contaminated	Use new TMB substrate with same Lot
	Evaporation of wells during incubations	Perform incubation steps with plate sealer in repeat assay
	Incorrect incubation times and/or temperatures	Follow the manual to repeat the assay
	TMB substrate is exposed to light	Use new TMB substrate

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