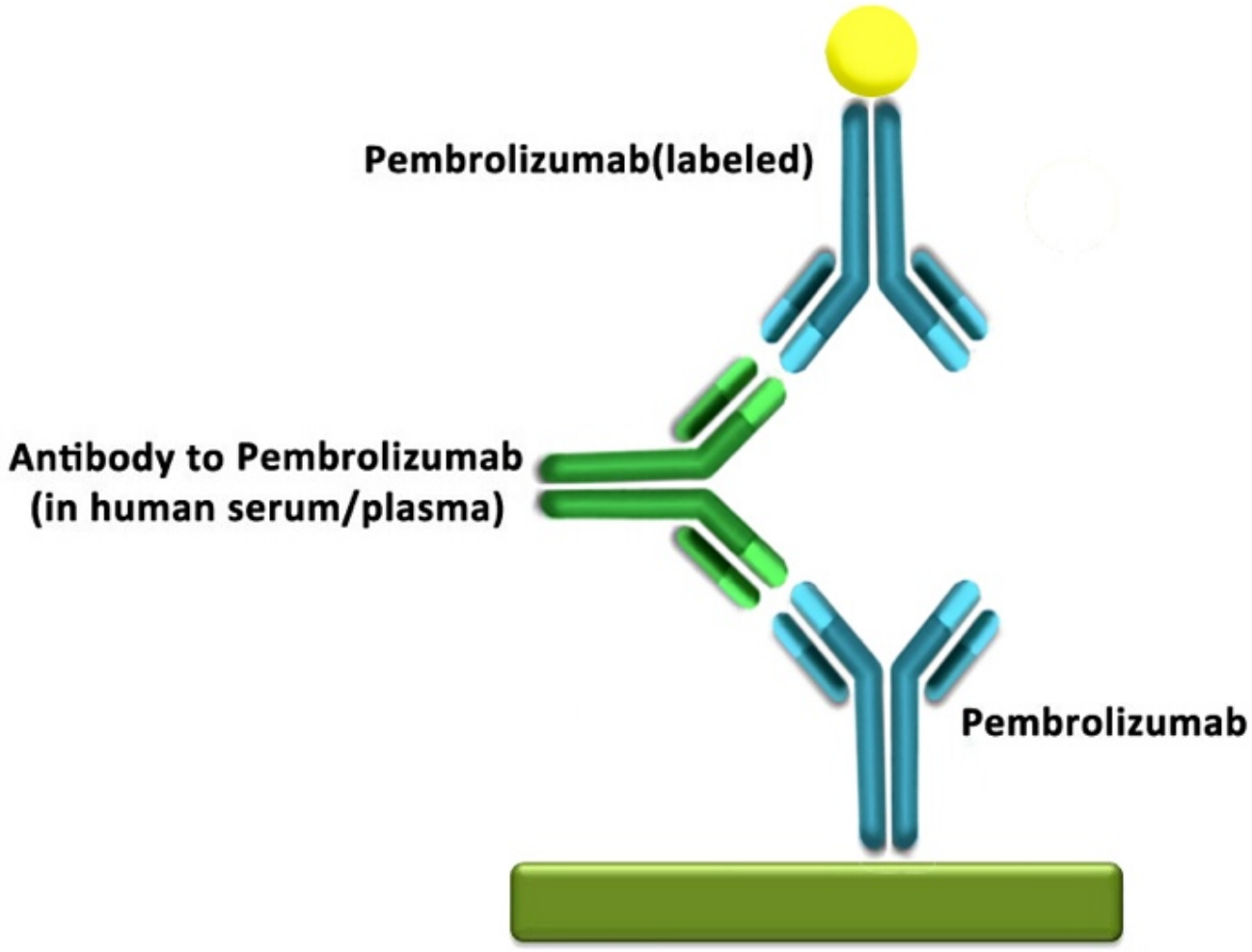
Pembrolizumab Immunogenicity ELISA Kit

Cat. No. L00706 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 Immunogenicity ELISA Kit is a sandwich enzyme-linked immunoassay which can be used for quantitative detection of antibodies against pembrolizumab in serum and plasma samples. During the first incubation period, the pembrolizumab coated on the wall of the microtiter wells captures the antibodies to pembrolizumab in patient serum and plasma. After washing away the unbound components from samples, a biotin-labeled pembrolizumab conjugate is added to each well and then incubated. After a second washing step

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and Horseradish peroxidase conjugated streptavidin is added and to react with the TMB substrate to develop a blue product in the solution. The reaction is stopped by adding stop solution which turns the color yellow and this can be read at 450 nm by a Microtiter plate reader. The intensity of the reaction color is directly proportional to the concentration of antibodies to pembrolizumab in samples.

II. KEY FEATURES

Featrure	Specification
Sensitivity	0.2056ng/ml
Detection Range	1.5625-100ng/ml
Test Samples	Human serum/plasma(EDTA), mouse serum,rat serum/plasma(heparin),rabbit serum/plasma(heparin)
Conveniency	All reagents and buffers for test are provided complete the test within 2.5 hours

III. KIT CONTENTS

- Reagents and buffers for keyruda detection.

Component	Quantity	Part No.
Capture Plate	1 plate (8 wells x 12 strips)	706-80
Biotin conjugate	12 mL	706-20
Streptavidin-HRP	12 mL	706-30
Reactive control (10μg/mL)	50 μL	706-10
Sample Dilution Buffer	60 mL	706-60
20 × Wash Solution	40 mL	706-70
TMB Substrate	12 mL	706-40
Stop Solution	6 mL	706-50
Plate Sealer	2 pieces	N/A
User Manual	1 copy	N/A

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.

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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.

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.

Reactive Control Preparation

- The kit provides *Reactive control* 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 *Control stock* and 990μL of *Sample Buffer* into the tube labeled with '100 ng/mL'and vortex it.
3. Pipette 500μLof Sample Buffer into the rest of the empty tubes.
4. Pipette 500μLof 100ng/mL of control solution to the tube labeled with '50ng/mL' and vortex it to make the control be 50ng/mL.
5. Similarly, prepare the rest of the control series (25，12.5，6.25，3.125，1.5625ng/mL).

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Samples preparation

Perform preliminary experiments to determine the optimum detection sample dilution.

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

Serum: Use a blood separator tube and allow the sample to clot for 30min. Centrifuge for 10min at 1000 x g. Run the assay immediately, otherwise aliquot and store the samples below -20℃. Avoid repeated freeze-thaw cycles. Serum samples usually require a 10-fold dilution.

Plasma: Treat the blood with EDTA or heparin as an anticoagulant. Centrifuge for 10min at 1000 x g within 30min for plasma collection. Run the assay immediately. Otherwise aliquot and store the samples blow -20℃.

Avoid repeated freeze-thaw cycles. Plasma samples usually require a 10-fold dilution.

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

	Reactive Control 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 reaction controls 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.

Test Procedure

Reactive control and samples Incubation

1. Add 100μL of Reaction control 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.

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Biotin conjugate Incubation

1. Add 100μL of *Biotin conjugate* to all the wells.
2. Cover the plate with *Plate Sealer* and incubate at 37 °C for 60min.
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* for 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 the time 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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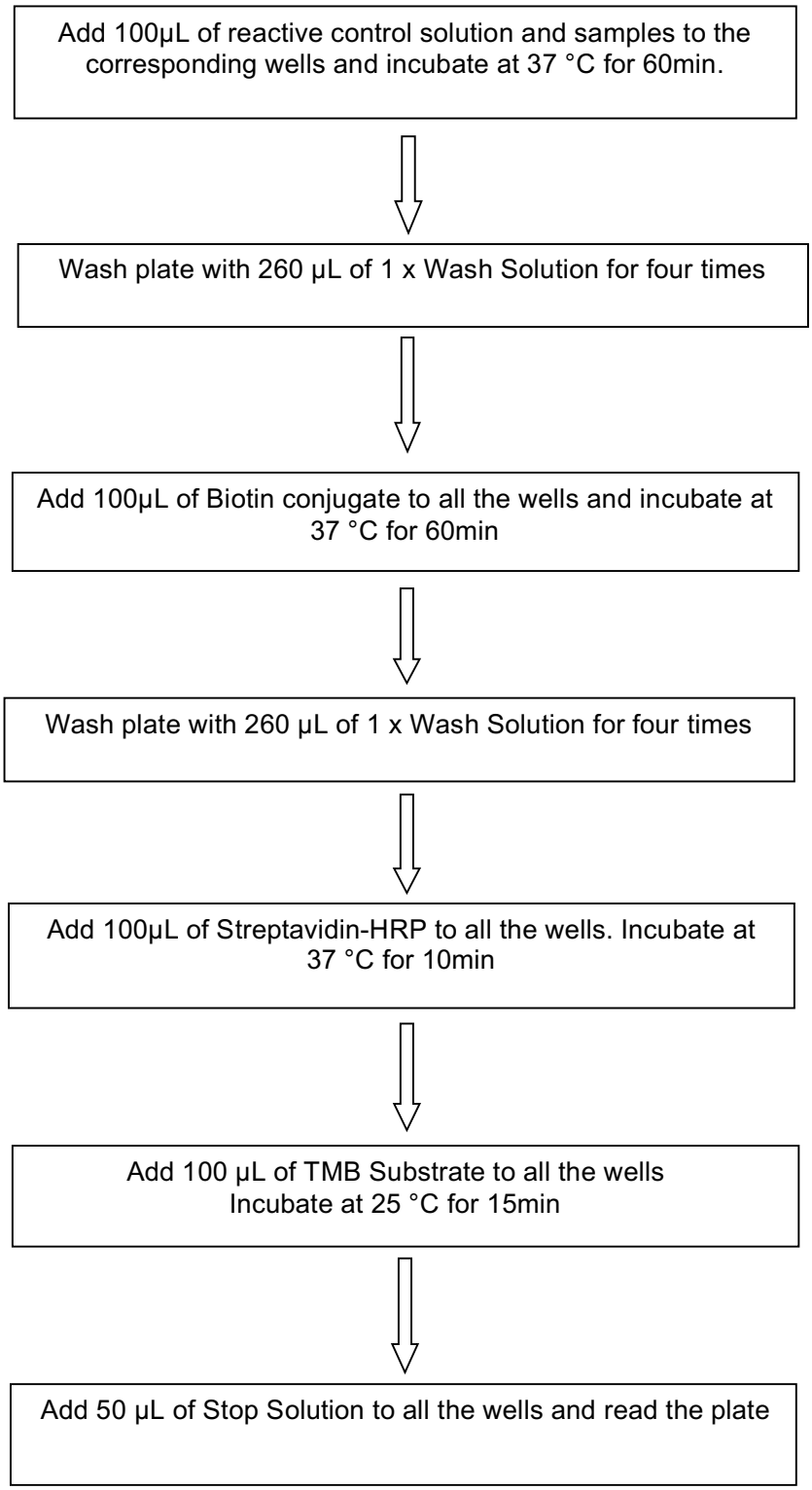
VII. ASSAY PROCEDUR SUMMARY



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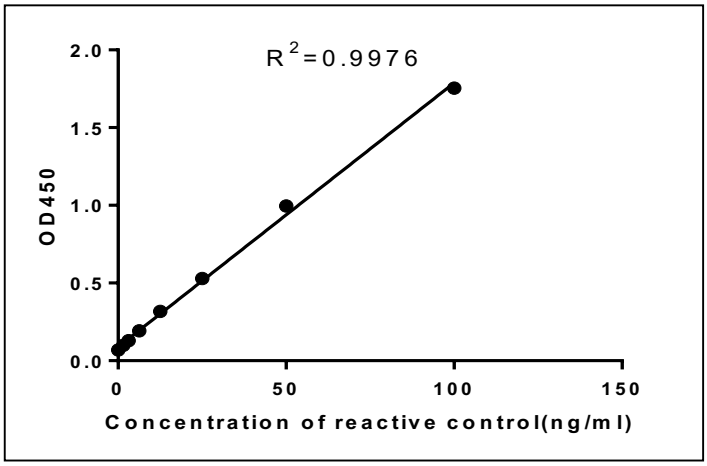
VIII. TYPICAL ASSAY DATA

The reaction control curve below was provided for demonstration only. Operator should set up control curve to precisely determine antibody to pembrolizumab each time.



Reactive Control	OD_{450}		
(ng/mL)	Duplicate 1	Duplicate 2	Average
100	1.774	1.735	1.755
50	0.99	1.003	0.997
25	0.515	0.545	0.53
12.5	0.323	0.312	0.318
6.25	0.194	0.19	0.192
3.125	0.131	0.127	0.129
1.5625	0.1	0.098	0.099
0	0.07	0.067	0.069

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 2.43%.

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 3.74%.

X. SENSITIVITY

The minimum detectable dose (MDD) of the assay is between 0.133-0.270 ng/mL. The mean MDD is 0.2056ng/mL.

XI. RECOVERY

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

Sample	Average recovery (%)	Range (%)
Human serum(n=5)	97.7	85.0-110.4
Human plasma(n=5)	97.8	85.8-111.2
Rabbit serum(n=5)	104.6	94.2-115.3
Rabbit plasma(n=5)	102.0	94.7-114.3
Mouse serum(n=5)	97.6	88.9-110.8
Rat serum(n=5)	99.5	87.5-111.2
Rat plasma(n=5)	92.9	86.7-105.4

XII. TROUBLESHOOTING

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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
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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