Datasheet for ABIN6969788

PYCARD ELISA Kit

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Overview

Quantity:	96 tests
Target:	PYCARD
Reactivity:	Human
Method Type:	Sandwich ELISA
Detection Range:	78.125 pg/mL - 5000 pg/mL
Minimum Detection Limit:	78.125 pg/mL
Application:	ELISA

Product Details

Purpose:	For quantitative detection of PYCARD in serum, plasma, tissue homogenates.
Sample Type:	Plasma, Serum, Tissue Homogenate
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Specificity:	This assay has high sensitivity and excellent specificity for detection of PYCARD. No significant cross- reactivity or interference between PYCARD and analogues was observed. Note: Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between PYCARD and all the analogues, therefore, cross reaction may still exist.
Sensitivity:	46.875 pg/mL
Components:	• Pre-coated, ready to use 96-well strip plate • Plate sealer for 96 wells • Standard

Product Details

	• Sample/Standard Dilution Buffer • Biotin-labeled Antibody (Concentrated) • Antibody Dilution Buffer • HRP-Streptavidin Conjugate (SABC) • SABC Dilution Buffer • TMB Substrate • Stop Solution • Wash Buffer (25 x concentrate) • Instruction manual
Material not included:	1. Microplate reader (wavelength:450nm) 2. 37 °C incubator 3. Automated plate washer 4. Precision single and multi-channel pipette and disposable tips 5. Clean tubes and Eppendorf tubes 6. Deionized or distilled water

Target Details

Target:	PYCARD
Alternative Name:	Apoptosis-associated speck-like protein containing a CARD (PYCARD Products)
Background:	HASC, Caspase recruitment domain-containing protein 5, PYD and CARD domain-containing protein, Target of methylation-induced silencing 1
UniProt:	Q9ULZ3
Pathways:	Activation of Innate immune Response, Cellular Response to Molecule of Bacterial Origin, Regulation of Actin Filament Polymerization, Positive Regulation of Endopeptidase Activity, Activated T Cell Proliferation, Inflammasome

Application Details

Sample Volume:	100 µL
Plate:	Pre-coated
Protocol:	1. Wash plate 2 times before adding Standard, Sample (diluted at least 1/2 with Sample Dilution Buffer) and Control (blank) wells! 2. Add 100 µL standard or sample to each well and incubate for 90 minutes at 37 °C. 3. Aspirate and wash plates 2 times. 4. Add 100 µL Biotin-labeled antibody working solution to each well and incubate for 60 minutes at 37 °C.

5. Aspirate and wash plates 3 times.
6. Add 100 µL SABC Working Solution into each well and incubate for 30 minutes at 37 °C.
7. Aspirate and wash plates 5 times.
8. Add 90 µL TMB Substrate Solution. Incubate 10-20 minutes at 37 °C.
9. Add 50 µL Stop Solution. Read at 450nm immediately and calculation.

Reagent Preparation:

- Bring all reagents and samples to room temperature for 20 minutes before use.
- Wash Buffer: If crystals have formed in the concentrate, you can warm it with 40 °C water bath Concentrated Wash Buffer into 750 mL Wash Buffer with deionized or distilled water. Put unused solution back at 2-8 °C.
- Standards:
  1. Add 1 mL Sample Dilution Buffer into one Standard tube (labeled as zero tube), keep the tube at room temperature for 10 minutes and mix them thoroughly. Note: If the standard tube concentration higher than the range of the kit,please dilute it and labeled as zero tube.
  2. Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3 mLof the Sample Dilution Buffer into each tube. Add 0.3 mLof the above Standard solution (from zero tube) into 1st tube and mix them thoroughly. Transfer 0.3 mL from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3 mL from 2nd tube to 3rd tube and mix them thoroughly, and so on. Sample Dilution Buffer was used for the blank control. Note: It is best to use Standard Solutions within 2 hours.
- Preparation of Biotin-labeled Antibody Working Solution:
  Prepare it within 1 hour before experiment.
  1. Calculate required total volume of the working solution: 0.1ml/well x quantity of wells. (Allow 0.1-0.2 mLmore than the total volume.)
  2. Dilute the Biotin-detection antibody with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1 µL Biotin-labeled antibody into 99 µL Antibody Dilution Buffer.)
- Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution:
  Prepare it within 30 minutes before experiment.
  1. Calculate required total volume of the working solution: 0.1ml/well x quantity of wells. (Allow 0.1-0.2 mLmore than the total volume.)
  2. Dilute the SABC with SABC Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1 µL of SABC into 99 µL of SABC Dilution Buffer.)

Sample Preparation:

- It is recommended to use fresh samples without long storage, otherwise protein degradation and denaturationmay occur in these samples, leading to false results. Samples should therefore be stored for a short periodat 2 - 8 °C or aliquoted at -20 °C (≤1 month) or -80 °C (≤ 3 months). Repeated freeze-thawcycles should be avoided. Prior to assay, the frozen samples should be slowly thawed and centrifuged toremove precipitates.
- If the sample type is not specified in the instructions, a preliminary test is necessary to determinecompatibility with the kit.
- If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibilityof causing a deviation due to the introduced chemical substance.The recommended dilution factor is for reference only.

- Please estimate the concentration of the samples before performing the test. If the values are not in therange of the standard curve, the optimal sample dilution for the particular experiment has to be determined.Samples should then be diluted with PBS (pH =7.0-7.2).

Note:

The user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be making in pre-experiment. If samples with very high concentrations, dilute samples with PBS first and then dilute the samples with Sample Dilution. The matrix components in the sample will affect the test results, which it need to be diluted at least 1/2 with Sample Dilution Buffer before testing!

Assay Procedure:

Washing

Manual: Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350 µL wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material.

Automatic: Aspirate all wells, and then wash plate with 350 µL wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer shall be set for soaking 1 minute. (Note: set the height of the needles, be sure the fluid can be sipped up completely)

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37 °C. It is recommended to plot a standard curve for each test.

1. Set standard, test samples (diluted at least 1/2 with Sample Dilution Buffer), control (blank) wells on the pre-coated plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate. Wash plate 2 times before adding standard, sample and control (blank) wells!
2. Prepare Standards: Aliquot 100 µL of zero tube, 1sttube, 2ndtube, 3rdtube, 4thtube, 5thtube, 6thtube and Sample Dilution Buffer (blank) into the standard wells.
3. Add Samples: Add 100 µL of properly diluted sample into test sample wells.
4. Incubate: Seal the plate with a cover and incubate at 37 °C for 90 minutes.
5. Wash: Remove the cover and discard the plate content, and wash plate 2 times with Wash Buffer. Do NOT let the wells dry completely at any time.
6. Biotin-labeled Antibody: Add 100 µL Biotin-labeled antibody working solution into above wells (standard, test sample and blank wells). Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 37 °C for 60 minutes.

Application Details

7. Wash: Remove the cover, and wash plate 3 times with Wash Buffer, and let the Wash Buffer stay in the wells for 1-2 minutes each time.
8. HRP-Streptavidin Conjugate (SABC): Add 100 µL of SABC Working Solution into each well, cover the plate and incubate at 37 °C for 30 minutes.
9. Wash: Remove the cover and wash plate 5 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minutes each time.
10. TMB Substrate: Add 90 µL TMB Substrate into each well, cover the plate and incubate at 37 °C in dark within 10-20 minutes. (Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells.)
11. Stop: Add 50 µL Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.
12. OD Measurement: Read the O.D. absorbance at 450nm in Microplate Reader immediately after adding the stop solution.

Regarding calculation, (the relative O.D.450) = (the O.D.450 of each well) - (the O.D.450 of blank well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. It is recommended to use some professional software to do this calculation, such as Curve Expert 1.3 or 1.4.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Assay Precision:	Intra-Assay: CV<8% Inter-Assay: CV<10%
Restrictions:	For Research Use only

Handling

Storage:	4 °C,-20 °C
Storage Comment:	1. For unopened kit: All the reagents should be kept according to the labels on vials. The Reference Standard and the 96-well stripe plate should be stored at -20 °C upon receipt while the other reagents should be stored at 4 °C. 2. For used kit: When the kit is used, the remaining reagents need to be stored according to the above storage condition. Besides, please return the unused wells to the foil pouch containing the desiccant pack, and zip-seal the foil pouch.
Expiry Date:	6 months

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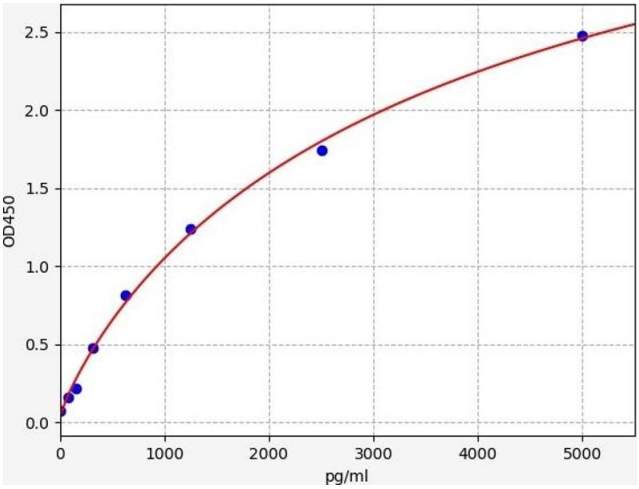

ELISA

Image 1. Typical standard curve