



(For scientific research use only, not for clinical diagnosis!)

**Human peptidyl arginine deiminase 4 (PAD4) quantitative
detection kit (ELISA) instruction manual Specifications:
48T/96T**

Please read the instructions carefully before use. If you have any questions,
please contact us through the following methods: Official hotline: 400-999-
8863 Technical phone: 18358180525 Email: Upingbio@163.com Company
website: www.upingbio.com For the specific shelf life, please see the outer
packaging label of the kit. Please use the kit within the shelf life.

When contacting us, please provide the product number and production date (see box label) so that we
can serve you more efficiently.

Kit performance Physical properties: Each liquid component is clear and transparent, with no precipitation or floc. Microplate aluminum foil bags should be vacuum packed without damage or leakage.

Standard curve linearity: Calibrator dose-response curve correlation coefficient r value, greater than or equal to 0.9900.

Precision: intra-batch variation coefficient CV% is less than 10%; inter-batch variation coefficient CV% is less than 15%.

Sensitivity: The lowest detectable dose is less than 0.1 ng/mL.

Recovery rate: The recovery rate is between 85%-115%.

Sensitivity: This kit recognizes native and recombinant human peptidyl arginine deiminase 4 (PAD4) and has no crossover with structural analogs.

Stability: Stored at 2°C-8°C, validity period is 6 months.

Detection range: 0.25 ng/mL – 8 ng/mL.

Purpose: Used to detect the concentration of human peptidyl arginine deiminase 4 (PAD4) in serum, plasma, cell culture supernatant and other samples.

Experimental principle This kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). In the microwell enzyme plate pre-coated with anti-human peptidyl arginine deiminase 4 (PAD4) antibody (solid-phase antibody), add human peptidyl arginine deiminase 4 (PAD4) calibrator and the sample to be tested, and then add anti-human peptidyl arginine deiminase 4 (PAD4) antibody (enzyme-labeled antibody). After incubation and sufficient washing, unbound components are removed and placed on the solid surface of the microplate. A sandwich complex of solid-phase antibody-antigen-enzyme-labeled antibody is formed. Add TMB substrate.

The substrate is catalyzed by HRP to produce a blue product. Under the action of the stop solution, it is finally converted into yellow. The absorbance (OD value) is measured at a wavelength of 450nm with a microplate reader. The absorbance (OD value) is the same as that to be measured. The concentration of human peptidyl arginine deiminase 4 (PAD4) in the sample is positively correlated. By fitting the calibrator curve, human peptidyl arginine deiminase 4 in the sample can be calculated

UpingBio technology Co.,Ltd

Website: www.upingbio.com

Official hotline: 400-999-8863

Supervision phone number:

(PAD4) concentration.

Kit components and storage: Store unopened kits at 2-8

degrees Celsius. Do not use expired kits.

Components	quantity	Main ingredients	Store after opening
Calibrator	0.3ml/tube	--	2-8°C14 days
coated microplate	96T/48T	Pre-coated solid phase antibodies	2-8°C14 days
HRP labeled antibodies	10mL	HRP-labeled detection antibodies	2-8°C180 days
sample diluent	6mL	--	2-8°C180 days
Substrate solution	10mL	0.1%TMB	2-8°C180 days
stop solution	6mL	2mol/L acidic solution	2-8°C180 days
20×concentrated washing liquid	25mL	0.05%Tween20	2-8°C180 days
manual	1 serving	--	--
Ziplock bag	1	--	--
self-adhesive	2 tablets	--	--

The concentrations of calibrators are: 8, 4, 2, 1, 0.5, 0.25 ng/mL.

Note: 1: Before use, please check whether the label and quantity of the reagents in the kit are consistent with the table.

2: If the components of the kit need to be used again, please ensure that they have not been contaminated since the last use. 3: If the enzyme plate is not used up in a single time, remember to seal it and store it at 2-8°C.

Prepare your own test equipment required for the test (not provided, but can assist in purchasing)

UpingBio technology Co.,Ltd

Website: www.upingbio.com

Official hotline: 400-999-8863

Supervision phone number:

1. Standard specification microplate reader.
2. Automatic plate washing machine.
3. Oscillator.
4. A series of adjustable pipettes and tips. When testing a large number of samples at one time, it is best to use a multi-channel pipette.

Restrictions on the kit: 1. For scientific research use only and not for clinical diagnosis.

2. Use within the validity period marked on the kit. Expired products must not be used.
3. Do not mix with kits or components from other manufacturers.
4. Use the sample diluent provided with the kit.
5. If the sample value is higher than the highest standard concentration value, please dilute the sample appropriately and then measure again.
6. Human anti-mouse and other heterophilic antibodies present in the sample to be tested will interfere with the test results. Please eliminate this factor before testing.
7. The test results obtained by other methods are not directly comparable to the test results of this kit.

Notes: 1) This kit is for in vitro research only and not for clinical diagnosis.

- 2) Please wear a lab coat and latex gloves for protection during the test. Especially when testing blood or other body fluid samples, please follow the national biological laboratory safety protection regulations.

3) Carry out incubation strictly according to the specified time and temperature to ensure accurate results. All reagents must reach room temperature 20-25°C before use. Store reagents refrigerated immediately after use.

4) Improper plate washing can lead to inaccurate results. Make sure to absorb as much liquid as possible from the wells before adding substrate. Do not

UpingBio technology Co.,Ltd

Website: www.upingbio.com

Official hotline: 400-999-8863

Supervision phone number:

Let the micropores dry out.

- 5) Eliminate residual liquid and fingerprints on the bottom of the plate, otherwise it will affect the OD value.
- 6) The substrate chromogenic solution should be colorless or very light in color.
- 7) Avoid cross-contamination of reagents and specimens to avoid erroneous results.
- 8) Avoid direct exposure to strong light during storage and incubation.
- 9) Equilibrate to room temperature before opening the sealed bag to prevent water droplets from condensing on the cold slats.
- 10) Any reaction reagents must not come into contact with bleaching solvents or strong gases emitted by bleaching solvents. Any bleaching ingredients will destroy the biological activity of the reagents in the kit.
- 11) The microplate reader used for detection needs to be equipped with a filter capable of detecting a wavelength of $450\pm 10\text{nm}$, and the optical density range is between 0-3.5. It is recommended to preheat 15 minutes in advance before use.
- 12) Do not mix or replace the reagents in this kit with reagents from other batch numbers or other sources.
- 13) The EP tubes and tips used in the test are single-use and are strictly prohibited from mixing.
- 14) Do not use expired reagents.

UpingBio technology Co.,Ltd

Website: www.upingbio.com

Official hotline: 400-999-8863

Supervision phone number:

Sample preparation and storage

The following lists only general guidelines for sample collection and preservation. During the collection and storage of all samples, sodium azide must not be used as a preservative. If the sample is not analyzed immediately, it should be aliquoted and stored frozen, and repeated freezing and thawing should be avoided.

Cell culture supernatant - centrifuge to remove precipitate, analyze immediately or aliquot and store frozen at -20°C.

Serum - Collect blood in a clean test tube, coagulate at room temperature for 30 minutes, centrifuge at 2000×g for 20 minutes, and collect serum. Analyze immediately or aliquot and store frozen at -20°C.

Plasma—anticoagulate with heparin, citrate, or EDTA, and centrifuge at 2000×g for 20 minutes at 2-8°C within 30 minutes of blood draw. To eliminate the influence of platelets, it is recommended to further centrifuge at 10,000 × g for 10 minutes at 2-8°C. Analyze immediately or aliquot and store frozen at -20°C.

Cell lysis buffer - For adherent cells, remove the culture medium and wash with PBS, normal saline or serum-free culture medium. Add an appropriate amount of lysis solution and pipet several times with a gun to fully contact the lysate and cells. Typically after 10 seconds, cells are lysed. For suspended cells, collect the cells by centrifugation and wash them with PBS, physiological saline or serum-free culture medium. Add an appropriate amount of lysis solution, blow the cells with a gun, and flick them with your fingers to fully lyse the cells. After full lysis, centrifuge at 10000-14000×g for 3-5 minutes and take the supernatant. Analyze immediately or aliquot and store frozen at -20°C.

Urine - Collect in sterile tubes and centrifuge at 2000×g for 20 minutes. Carefully collect the supernatant. If a precipitate forms, centrifuge again.

Reagent preparation 1. Before use, all components must be rewarmed for at least 120 minutes to ensure sufficient rewarming to room temperature.

2. Concentrated washing liquid: The concentrated washing liquid taken out from the refrigerator will produce crystals. This is a normal phenomenon. Heating in a water bath will completely dissolve the crystals. Dilute concentrated washing liquid and distilled water at 1:20, that is, add 1 part of concentrated washing liquid to 19 parts of distilled water.

UpingBio technology Co.,Ltd

Website: www.upingbio.com

Official hotline: 400-999-8863

Supervision phone number:

Operating procedures

Recommended sample dilution scheme: It is recommended that teachers conduct preliminary experiments to explore the optimal dilution ratio of samples before conducting formal experiments.

All reagents and components should be returned to room temperature first. It is recommended to perform duplicate wells for standards, quality control materials and samples.

1. Prepare the working solutions of various components of the kit according to the method described in the previous instructions.

2. Take out the required slats from the aluminum foil bag, seal the remaining slats in a ziplock bag and return it to the refrigerator.

Set up standard wells, 0-value wells, blank wells and sample wells. Add 50 μL of standards of different concentrations to each of the standard wells. Add 50 μL of sample diluent to the 0-value well. Do not add it to the blank well. Add 50 μL of the sample to be tested to the sample well.

3. In addition to the blank wells, add 100 μL of horseradish peroxidase (HRP)-labeled detection antibody to the standard wells, 0 value wells and sample wells.

4. Cover the reaction plate with sealing film and incubate it in a 37°C water bath or incubator for 60 minutes.

5. Uncover the sealing film, discard the liquid, pat dry on absorbent paper, fill each well with washing solution, let stand for 20 seconds, shake off the washing solution, pat dry on absorbent paper, repeat 5 times. If you use an automatic plate washer, please wash the plate according to the plate washer operating procedure. Adding a soaking program for 30 seconds can improve the detection accuracy.

After washing the plate and before adding substrate, pat the reaction plate dry on clean, lint-free paper.

6. Add 100 μL of TMB substrate to all wells. Cover the reaction plate with sealing film and incubate in a 37°C water bath or incubator for 15 minutes. 7. Add 50 μL of stop solution to all wells, and read the absorbance (OD value) of each well at a wavelength of 450 nm on a microplate reader.

UpingBio technology Co.,Ltd

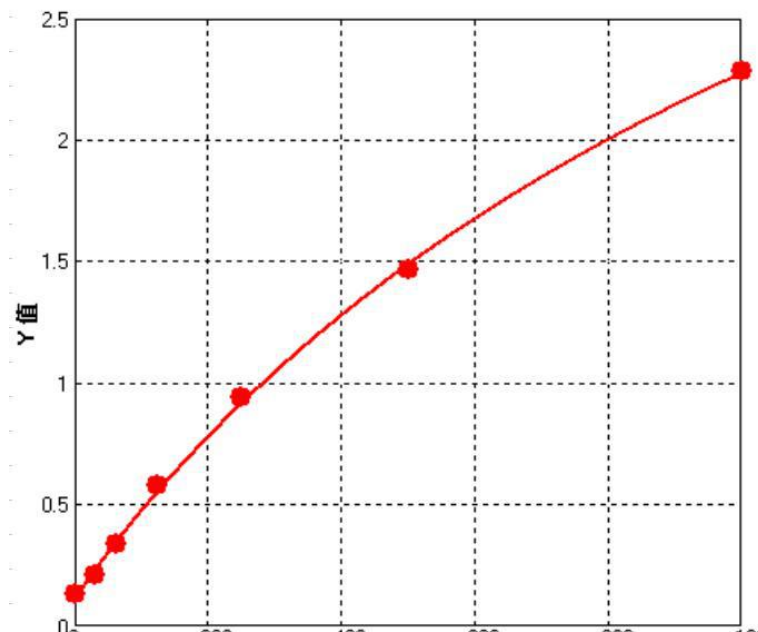
Website: www.upingbio.com

Official hotline: 400-999-8863

Supervision phone number:

Result calculation

9. Use the concentration of the standard substance as the abscissa and the corresponding absorbance (OD value) as the ordinate. Use computer software and four-parameter Logistic curve fitting (4-pl) to create a standard curve equation. Through the absorbance (OD value) of the sample value), use the equation to calculate the concentration value of the sample. [Calculation using ELISA Calc software]
10. If the sample is diluted, the concentration value measured by the above method must be multiplied by the dilution factor to obtain the final concentration of the sample.



(Schematic diagram, for reference only)

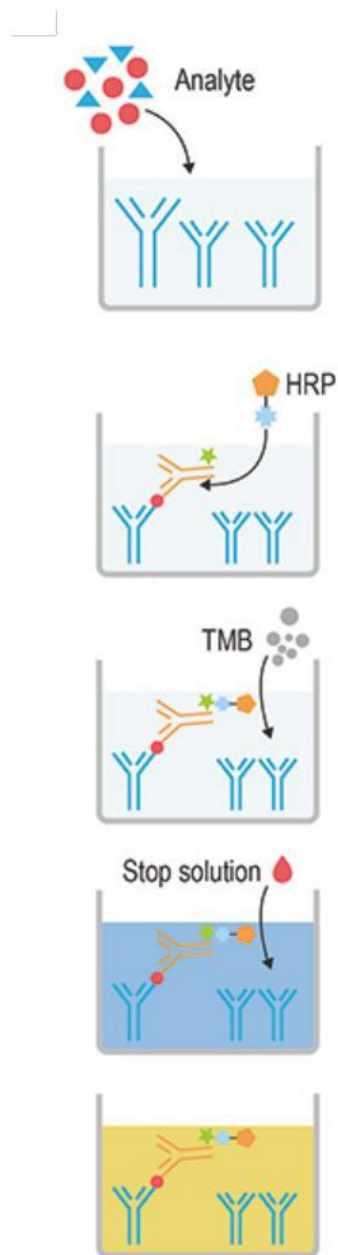
UpingBio technology Co.,Ltd

Website: www.upingbio.com

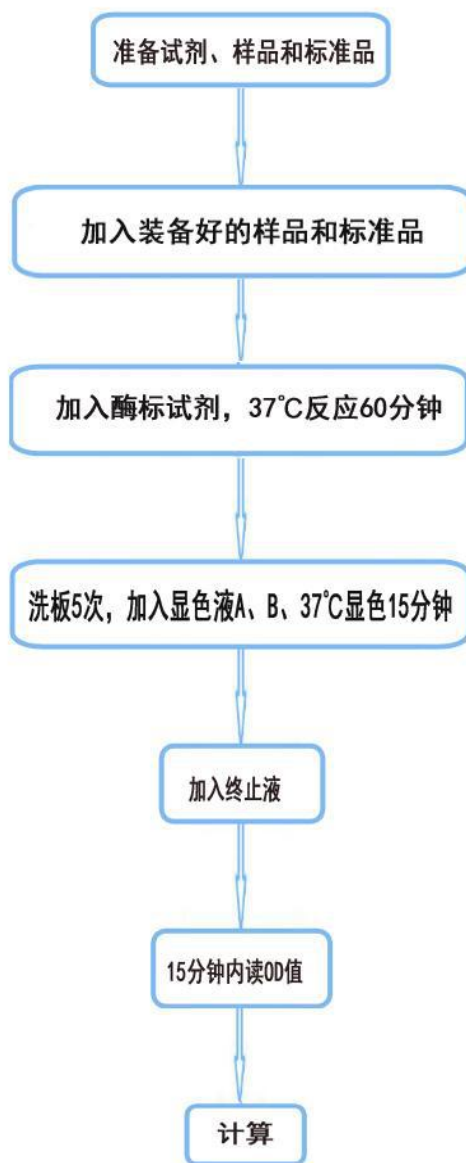
Official hotline: 400-999-8863

Supervision phone number:

[Operation Summary]



操作程序



UpingBio technology Co.,Ltd

Website: www.upingbio.com

Official hotline: 400-999-8863

Supervision phone number:

[Problem Analysis] If the experimental results are not good, please take pictures of the color development results in time, save the experimental data, keep the used laths and unused reagents, and then contact our company's technical support to solve the problem for you. At the same time, you can also refer to the following information: [Questions and Answers]

Problem Description	Possible Causes	Corresponding countermeasures Corresponding countermeasures
standard curve gradient difference	Incorrect liquid aspiration or	Check pipettes and tips
	Equilibration time is too short	Ensure sufficient balancing time
	Incomplete washing	Ensure the washing time and number of washes and the amount of liquid added to each hole
Very weak or colorless	Incubation time too short	Ensure adequate incubation time
	Experimental temperature is incorrect	Use recommended experimental temperatures
	Insufficient reagent volume or missing addition	Check the liquid aspirating and adding process to ensure that all reagents are added in order and in
	Incorrect dilution	
Enzyme label inactivation or substrate failure	Mix enzyme conjugate and substrate and check by rapid color development	
Reading value is low	Microplate reader settings are incorrect	Check the wavelength and filter
		Turn on the microplate reader and preheat it in advance
Large coefficient of variation	Adding fluid incorrectly	Check the filling situation
High background value	The working concentration of the	Use the recommended dilution
	Incomplete washing of enzyme plate	Ensure that each step of cleaning is complete; if using an automatic plate washer, please check whether all outlets are blocked;
	The lotion is contaminated	Prepare fresh lotion
Low sensitivity	Improper storage of ELISA kits	Store relevant reagents according to
	Not terminated before reading	Stop solution should be added to

UpingBio technology Co.,Ltd

Website: www.upingbio.com

Official hotline: 400-999-8863

Supervision phone number: