



Mouse PAR2 ELISA Kit

User Manual

Catalog # CEK0063

(Version 1.1A)

Sandwich Enzyme-Linked Immunosorbent Assay for Quantitative
Detection of Mouse PAR2 Concentrations in Tissue homogenates,
Cell lysates.

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

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I. INTRODUCTION

Receptor for trypsin and trypsin-like enzymes coupled to G proteins. Its function is mediated through the activation of several signaling pathways including phospholipase C (PLC), intracellular calcium, mitogen-activated protein kinase (MAPK), I-kappaB kinase/NF-kappaB and Rho. Can also be transactivated by cleaved F2R/PAR1. Involved in modulation of inflammatory responses and regulation of innate and adaptive immunity, and acts as a sensor for proteolytic enzymes generated during infection. Generally is promoting inflammation. Can signal synergistically with TLR4 and probably TLR2 in inflammatory responses and modulates TLR3 signaling. Has a protective role in establishing the endothelial barrier; the activity involves coagulation factor X. Proposed to have a bronchoprotective role in airway epithelium, but also shown to compromise the airway epithelial barrier by interrupting E-cadherin adhesion. Involved in the regulation of vascular tone; activation results in hypotension presumably mediated by vasodilation. Associates with a subset of G proteins alpha subunits such as G alpha-q, G alpha-11, G alpha-14, G alpha-12 and G alpha-13, but probably not with G(o) alpha, G(i) subunit alpha-1 and G(i) subunit alpha-2. However, according to PubMed:21627585 can signal through G(i) subunit alpha. Believed to be a class B receptor which internalizes as a complex with arrestin and traffic with it to endosomal vesicles, presumably as desensitized receptor, for extended periods of time. Mediates inhibition of TNF-alpha stimulated JNK phosphorylation via coupling to G alpha-q/11; the function involves dissociation of RIPK1 and TRADD from TNFR1. Mediates phosphorylation of nuclear factor NF-kappa-B RELA subunit at 'Ser-536'; the function involves IKBKB and is predominantly independent of G proteins. Involved in cellular migration. Involved in cytoskeletal rearrangement and chemotaxis through beta-arrestin-promoted scaffolds; the function is independent of G alpha-q/11 and involves promotion of cofilin dephosphorylation and actin filament severing. Induces redistribution of COPS5 from the plasma membrane to the cytosol and activation of the JNK cascade is

mediated by COPS5. Involved in the recruitment of leukocytes to the sites of inflammation and is the major PAR receptor capable of modulating eosinophil function such as proinflammatory cytokine secretion, superoxide production and degranulation. During inflammation promotes dendritic cell maturation, trafficking to the lymph nodes and subsequent T-cell activation. Involved in antimicrobial response of innate immune cells; activation enhances phagocytosis of Gram-positive and killing of Gram-negative bacteria. Acts synergistically with interferon-gamma in enhancing antiviral responses. Implicated in a number of acute and chronic inflammatory diseases such as of the joints, lungs, brain, gastrointestinal tract, periodontium, skin, and vascular systems, and in autoimmune disorders.

II. ASSAY PRINCIPLES

Cohesion Bioscience Mouse PAR2 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of Mouse PAR2 in Tissue homogenates, Cell lysates. This assay employs an antibody specific for Mouse PAR2 coated on a 96-well plate. Standards and samples are pipetted into the wells and PAR2 present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-Mouse PAR2 antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of PAR2 bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

III. KIT COMPONENTS

Component	Volume
96-well Plate Coated with Anti-Mouse PAR2 Antibody	12 x 8 Strips
Mouse PAR2 Standard	10 ng x 2
Biotin-Labeled Detection Antibody (100X)	120 µl
Streptavidin-HRP (100X)	120 µl
Standard/Sample Diluent	30 ml
Detection Antibody Diluent	12 ml
Streptavidin-HRP Diluent	12 ml
Wash Buffer (20X)	30 ml
TMB Substrate Solution	12 ml
Stop Solution	12 ml
Plate Adhesive Strips	3 Strips
Technical Manual	1 Manual

IV. STORAGE AND STABILITY

All kit components are stable at 2 to 8 °C. Standard (recombinant protein) should be stored at -20 °C or -80 °C (recommended at -80 °C) after reconstitution. Opened Microplate Wells or reagents may be store for up to 1 month at 2 to 8 °C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.

Note: the kit can be used within one year if the whole kit is stored at -20 °C. Avoid repeated freeze-thaw cycles.

V. MATERIALS REQUIRED BUT NOT PROVIDED

1. Microplate reader capable of measuring absorbance at 450 nm.
2. Adjustable pipettes and pipette tips to deliver 2 µl to 1 ml volumes.
3. Adjustable 1-25 ml pipettes for reagent preparation.
4. 100 ml and 1 liter graduated cylinders.
5. Absorbent paper.
6. Distilled or deionized water.
7. Computer and software for ELISA data analysis.
8. Tubes to prepare standard or sample dilutions.

VI. HEALTH AND SAFETY PRECAUTIONS

1. Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin. Please carefully review the MSDS for each reagent before conducting the experiment.
2. Stop Solution contains 2 N Sulfuric Acid (H_2SO_4) and is an extremely corrosive agent. Please wear proper eye, hand and face protection when handling this material. When the experiment is finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop Solution prior to disposing the plate.

VII. REAGENT PREPARATION

1. Sample Preparation

Store samples to be assayed within 24 hours at 2-8°C. For long-term storage, aliquot and freeze samples at -20°C. Avoid repeated freeze-thaw cycles.

Cell culture supernates: Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

Serum: Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1000 X g for 15 minutes. Analyze the serum immediately or aliquot and store samples at -20°C.

Plasma: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1500 X g within 30 minutes of collection. Assay immediately or aliquot and store samples at -20°C.

Cell Lysates: Collect cells and rinse cells with PBS. Homogenize and lyse cells thoroughly in lysate solution. Centrifuge cell lysates at approximately 10000 X g for 5 minutes to remove debris. Aliquots of the cell lysates were removed and assayed.

Bone Tissue: Extract demineralized bone samples in 4 M Guanidine-HCl and protease inhibitors. Dissolve the final sample in 2 M Guanidine-HCl.

Tissue Homogenates: The preparation of tissue homogenates will vary depending upon tissue type. Rinse tissue with 1X PBS to remove excess blood, homogenized in 20 mL of 1X PBS and stored overnight at $\leq -20^{\circ}\text{C}$. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g. The supernate was removed immediately and assayed.

Alternatively, aliquot and store samples at $\leq -20^{\circ}\text{C}$.

Note: Some lysis buffer, such as RIPA can not be used. Some components will affect the binding.

Urine: Urinary samples should be cleared by centrifugation and then can be used directly without dilution. Storage at -20°C.

2. Mouse PAR2 Standard Preparation

Reconstitute the lyophilized Mouse PAR2 Standard by adding 1 ml of Standard/Sample Diluent to make the 10 ng/ml standard stock solution. Allow solution to sit at room temperature for 5 minutes, then gently vortex to mix completely. Use within one hour of reconstituting. Two tubes of the standard (10 ng per tube) are included in each kit. Use one tube for each experiment.

Perform 2-fold serial dilutions of the top standards to make the standard curve within the range of this assay (0.156 ng/ml - 10 ng/ml) as below. Standard/Sample Dilution Buffer serves as the zero standard (0 ng/ml).

Standard	Add	Into
10 ng/ml		
5 ng/ml	500 µl of the Standard (10 ng/ml)	500 µl of the Standard/Sample Diluent
2.5 ng/ml	500 µl of the Standard (5 ng/ml)	500 µl of the Standard/Sample Diluent
1.25 ng/ml	500 µl of the Standard (2.5 ng/ml)	500 µl of the Standard/Sample Diluent
0.625 ng/ml	500 µl of the Standard (1.25 ng/ml)	500 µl of the Standard/Sample Diluent
0.312 ng/ml	500 µl of the Standard (0.625 ng/ml)	500 µl of the Standard/Sample Diluent
0.156 ng/ml	500 µl of the Standard (0.312 ng/ml)	500 µl of the Standard/Sample Diluent
0 ng/ml	1 ml of the Standard/Sample Diluent	

Note: The standard solutions are best used within 2 hours. The 10 ng/ml standard solution should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

3. Biotin-Labeled Detection Antibody Working Solution Preparation

The Biotin-Labeled Detection Antibody should be diluted in 1:100 with the Detection Antibody Diluent and mixed thoroughly. The solution should be prepared no more than 2 hours prior to the experiment.

4. Streptavidin-HRP Working Solution Preparation

The Streptavidin-HRP should be diluted in 1:100 with the Streptavidin-HRP Diluent and mixed thoroughly. The solution should be prepared no more than 1 hour prior to the experiment.

5. Wash Buffer Working Solution Preparation

Pour entire contents (30 ml) of the Wash Buffer Concentrate into a clean 1,000 ml graduated cylinder. Bring final volume to 600 ml with glass-distilled or deionized water (1:20).

VIII. ASSAY PROCEDURE

The Streptavidin-HRP Working Solution and TMB Substrate Solution must be kept warm at 37°C for 30 minutes before use. When diluting samples and reagents, they must be mixed completely and evenly. Standard detection curve should be prepared for each experiment. The user will decide sample dilution fold by crude estimation of protein amount in samples.

1. Add 100 µl of each standard and sample into appropriate wells.
2. Cover well and incubate for 90 minutes at room temperature or overnight at 4°C with gentle shaking.
3. Remove the cover, discard the solution and wash plate 3 times with Wash Buffer Working Solution, and each time let Wash Buffer Working Solution stay in the wells for 1 - 2 minutes. Blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.
4. Add 100 µl of Biotin-Labeled Detection Antibody Working Solution into each well and incubate the plate at 37°C for 60 minutes.
5. Wash plate 3 times with Wash Buffer Working Solution, and each time let Wash Buffer Working Solution stay in the wells for 1 - 2 minutes. Discard the Wash Buffer Working Solution and blot the plate onto paper towels or other absorbent material.
6. Add 100 µl of Streptavidin-HRP Working Solution into each well and incubate the plate at 37°C for 45 minutes.
7. Wash plate 5 times with Wash Buffer Working Solution, and each time let wash buffer stay in the wells for 1 - 2 minutes. Discard the wash buffer and blot the plate onto paper towels or other absorbent material.
8. Add 100 µl of TMB Substrate Solution into each well and incubate plate at 37°C in dark for 30 minutes.
9. Add 100 µl of Stop Solution into each well. The color changes into yellow immediately.

10. Read the O.D. absorbance at 450nm in a microplate reader within 30 minutes after adding the Stop Solution.

For calculation, (the relative O.D.450) = (the O.D.450 of each well) - (the O.D.450 of Zero 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 concentration of the samples can be interpolated from the standard curve.

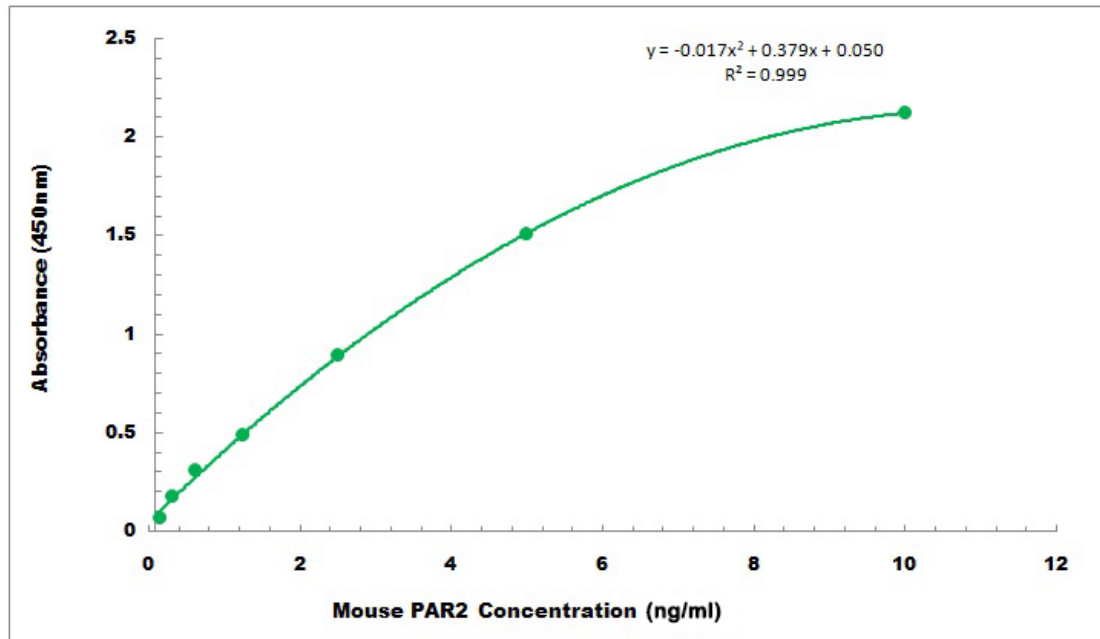
Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

IX. ASSAY PROCEDURE SUMMARY



X. TYPICAL DATA

The standard curve is for demonstration only. A standard curve must be run with each assay.



XI. SENSITIVITY

The minimum detectable dose of Mouse PAR2 is typically less than 53 pg/ml.

XII. SPECIFICITY

Mouse PAR2 ELISA Kit allows for the detection and quantification of endogenous levels of natural and/or recombinant Mouse PAR2 proteins within the range of 0.156 ng/ml - 10 ng/ml.

XIII. CROSS REACTIVITY

No detectable cross-reactivity with other relevant proteins.

XIV. REFERENCES

XV. TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
High signal and background in all wells	• Insufficient washing	• Increase number of washes • Increase time of soaking between in wash
	• Too much Streptavidin-HRP	• Check dilution, titration
	• Incubation time too long	• Reduce incubation time
	• Development time too long	• Decrease the incubation time before the stop solution is added
No signal	• Reagent added in incorrect order, or incorrectly prepared	• Review protocol
	• Standard has gone bad (If there is a signal in the sample wells)	• Check the condition of stored standard
	• Assay was conducted from an incorrect starting point	• Reagents allows to come to 20 - 30 °C before performing assay
Too much signal-whole plate turned uniformly blue	• Insufficient washing-unbound Streptavidin-HRP remaining	• Increase number of washes Carefully
	• Too much Streptavidin-HRP	• Check dilution
	• Plate sealer or reservoir reused, resulting in presence of residual Streptavidin-HRP	• Use fresh plate sealer and reagent reservoir for each step
Standard curve achieved but poor discrimination between point	• Plate not developed long enough	• Increase substrate solution incubation time
	• Improper calculation of standard curve dilution	• Check dilution, make new standard curve
No signal when a signal is expected, but standard curve looks fine	• Sample matrix is masking detection	• More diluted sample Recommended
Samples are reading too high, but standard curve is fine	• Samples contain protein levels above assay range	• Dilute samples and run Again
Edge effect	• Uneven temperature around work surface	• Avoid incubating plate in areas where environmental conditions vary • Use plate sealer

XVI. TECHNICAL SUPPORT

For troubleshooting, information or assistance, please go online to www.cohesionbio.com or contact us at techsupport@cohesionbio.com

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XVII. NOTES