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SIGNALCHEM BIOTECH

PAD4, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog # P312-310DG

Lot # Z5000-15

Product Description

Recombinant full-length human PAD4 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is BC025718.

Alternative Name(s)

PADI4; PAD; PDI4; PDI5

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

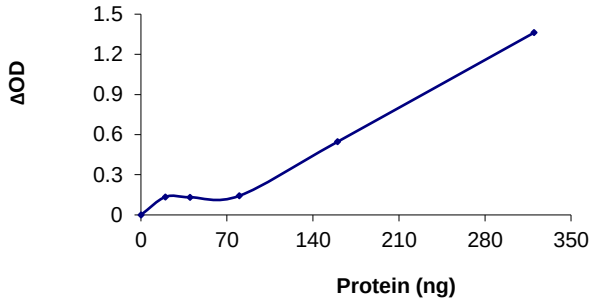
Scientific Background

PAD4 is a member of the peptidyl arginine deiminase family of enzymes, which catalyze the post-translational deimination of proteins by converting arginine residues into citrullines in the presence of calcium ions (1). PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps (NETs) (2). PAD4 play a role in granulocyte and macrophage development leading to inflammation and immune response. PAD4 also mediates the gene expression by regulating arginine methylation and citrullination in histones (3).

References

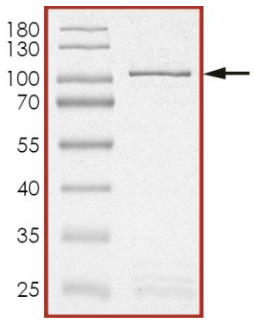
1. Nakashima, K. et.al: Molecular characterization of peptidylarginine deiminase in HL-60 cells induced by retinoic acid and 1-alpha,25-dihydroxyvitamin D(3). J. Biol. Chem. 274: 27786-27792, 1999.
2. Li, P.et.al: PAD4 is essential for antibacterial innate immunity mediated by neutrophil extracellular traps. J. Exp. Med. 207: 1853-1862, 2010.
3. Wang, Y. et.al: Human PAD4 regulates histone arginine methylation levels via demethylimination. Science 306: 279-283, 2004.

Specific Activity



The specific activity of PAD4 was determined to be **3.8 nmol/min/mg** as per activity assay protocol.

Purity



The purity of PAD4 was determined to be **>90%** by densitometry.
Observed MW **~105 kDa**.
Calculated MW **~100 kDa**.

Related Products:

P310BG-863	PAD4 Activity Detection Kit
P312-863	PAD Activity Detection Kit
P312-310G	PAD1, Active
P312-318BG	PAD2, Active
P312-310BG	PAD2, Active (mouse)
P312-310CG	PAD3, Active
P312-30FG	PAD6 Protein
P312-37C	PAD Cocktail, Active

PAD4, Active

Catalog #	P312-310DG
Specific Activity	3.8 nmol/min/mg
Lot #	Z5000-15
Purity	>90%
Concentration	0.1 μg/μl
Stability	1yr at –70°C from date of shipment

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https://www.sinobiological.com/category/kinase-enzyme-signalchem

Activity Assay Protocol

Reaction Components

PAD4, Active (Catalog #: P312-310DG)

10µl active PAD4 (1µg of total PAD4) diluted with 20µl of PAD Buffer (Note: these are suggested working dilutions and it is recommended that the researcher perform appropriate dilutions for optimal results).

PAD Activity Detection Kit (Cat#: P312-863)

Detection Antibody, 500X
NeutrAvidin™ Coated Plate
Chromogenic Substrate
PAD Buffer
Stop Solution
Wash Buffer, 20X
PAD Substrate, Lyophilized
Trypsin, 10mg/ml
Trypsin Buffer
DTT, 1M

Assay Protocol

Step 1. Prepare coating solution by diluting PAD Substrate in 1X wash buffer to 0.5 ug/ml. For standard curve, perform 8-point serial dilution in 1X Wash buffer.

Step 2. Place the NeutrAvidin coated strips onto an ELISA plate frame. Wash the wells 3X with wash buffer. Invert plate and blot dry.

Step 3. Add 100µL of the substrate coating solution per well. Incubate at room temperature for 1 hour.

Step 4. Decant solution from the plate. Wash the wells 4X with 1X wash buffer. After last wash, invert plate and blot dry.

Step 5. Add 50µL of the test sample, enzyme standard or PAD buffer to each well. Cover all wells with a plate sealer and incubate at 37°C for the desired time period (usually 20-60 minutes depending on the activity present in the sample).

Step 6. Repeat Step 4 as described.

Step 7. Add 100µL trypsin buffer to each well and aspirate by pipetting.

Step 8. Prepare trypsin digestion solution by diluting the stock 1000-fold in trypsin buffer.

Step 9. Add 100µL trypsin digestion reagent in all wells except for the standard curve (use trypsin buffer only). Cover all wells with a plate sealer and incubate at 37°C for 1 hour.

Note: The duration of trypsin digestion can be shortened if using a higher concentration of trypsin. It is advisable to obtain a trypsin dose response to determine the optimal concentration of trypsin if a different digestion time is used.

Step 10. Repeat Step 4 as described.

Step 11. Dilute the Detection antibody 500X in 1X wash buffer.

Step 12. Add 100µL detection antibody to each well. Incubate at room temperature for 1 hour.

Step 13. Repeat Step 4 as described.

Step 14. Warm up the Chromogenic Substrate to room temperature. Add 50µL Chromogenic substrate to each well. Incubate at room temperature for 20 minutes and protected from light.

Step 15. Add 25µL stop solution to each well. The color in the wells should change from blue to yellow. If the color change does not appear uniform, gently tap the plate to ensure thorough mixing.

Step 16. Within 30 minutes, read the optical density (OD) of all assay wells at 450nm and 540nm in a microplate reader.

Step 17. Calculation of Specific Activity:

A standard curve that correlates the OD with the amount of citrullinated substrate must be generated in the same experiment the sample is assayed.

- Subtract OD value measured at 540 nm from that measured at 450 nm for each assay well. This subtraction will correct for optical imperfections in the plates. Readings made directly at 450 nm without correction may be higher and less accurate.
- Subtract the average OD value of the blank wells from the values of those wells that have PAD reactions.
- Using the standard curve and OD value for a PAD sample, determine concentration or quantity of citrullinated substrate.
- Calculate activity using the equation below:

$$\text{Specific Activity (nmol/min/mg)} = \frac{\text{Substrate Conc. (µg / mL)} \times \text{Coating Solution Vol (µL)}}{\text{Substrate Mw (Da)} \times \text{Reaction Time (min)} \times \text{Protein Amount (mg)}}$$

where the Substrate Mw is 3687 Da, Protein Amount is the mass of total protein in a sample containing PAD activity.

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SAFETY DATA SHEET

Article 1 – Product Identification

Product Name: PAD4, Active **Catalog # P312-310DG**

This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Manufacturer's Name:	SignalChem Biotech Inc.
Street Address:	*110-13120 Vanier Place*
City, Prov. Postal Code:	*Richmond, BC, V6V 2J2*
Fax:	604-232-4601
EMERGENCY PHONE:	604-232-4600

Article 2 - Hazard Identification

- **WHMIS Classification:** Not WHMIS controlled.
- **GHS classification:** Skin irritation (Category 3); Eye irritation (Category 2B).
- **Hazard Pictograms:** none.
- **Signal words:** Warning.
- **Hazard statements:** Causes mild skin irritation (H316); Causes eye irritation (H320).
- **Precautionary statements:** IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. (P305 + P351 + P338).
- **Other hazards:** none known.

Article 3 – Composition/Information on Ingredients

Chemical Characterization: Mixtures.
Description: This product consists of the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Glycerol	Glycerol	56-81-5	≤25%
NaCl	Sodium chloride	7647-14-5	≤1.753%
Tris-HCl; Tris (hydroxymethyl) aminomethane hydrochloride	2 – Amino – 2 - (hydroxymethyl) propane - 1, 3 - diol hydrochloride	1185-53-1	<0.8%
Glutathione	Glutathione	70-18-8	0.307%
Protein		No data available	≤0.02%
DTT; Dithiothreitol	(R*,R*)-1,4-Dimercaptobutane-2,3-diol	3483-12-3	≤0.0154%
EDTA	Ethylenediaminetetraacetic acid	6381-92-6	0.0037%
PMSF; Phenylmethanesulfonyl fluoride	a-Toluenesulphonyl fluoride	329-98-6	≤0.002%

Article 4 – First-aid Measures

- **General information:** Consult a physician by providing the SDS.
- **After inhalation:** Breathe in fresh air. If cannot breathe, give artificial respiration and consult a physician.
- **After skin contact:** Immediately wash with soap and plenty of water and rinse thoroughly. Consult a physician.
- **After eye contact:** Rinse opened eyes with plenty of water for at least 15 minutes. Consult a physician.
- **After swallowing:** rinse the mouth with plenty of water and consult a physician.

Article 5 - Fire-fighting Measures

- **Suitable extinguishing media:** Use water spray, extinguishing powder, carbon dioxide, or other appropriate measure that is suitable to the environment.
- **Specific hazards arising from the substance or mixture:** None known.
- **Special protective equipment and precautions for fire-fighters:** Self-contained breathing apparatus if necessary.

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Article 6 – Accidental Release Measures

- **Personal precautions, protective equipment and emergency procedures:** Apply standard laboratory practices and personal protective equipment. Avoid breathing vapors, mist, or gas. Ensure adequate ventilation.
- **Environmental precautions:** Do not allow to enter drains.
- **Methods and materials for containment and cleaning up:** Absorb on sand or vermiculite and place in closed containers for disposal.

Article 7 - Handling and Storage

- **Precautions for sate handling:** Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.
- **Conditions for safe storage:** Store in a dry and well-ventilated place in -70 °C. Keep container upright and tightly closed.

Article 8 - Exposure Controls/Personal Protection

- **Components with limit monitoring values at workplace:**
  Glycerol (CAS-No: 56-81-5)

Values	Control parameters	Regulations
TWA	10 mg/m³ for mist	British Columbia, Canada
TWA	3 mg/m³ for respirable mist	British Columbia, Canada
TWA	10 mg/m³	Alberta, Canada
TWAEV	10 mg/m³	Ontario, Canada
TWAEV	10 mg/m³	Quebec, Canada
TWA	10 mg/m³	USA

- **Appropriate engineering controls:**
  Apply adequate ventilation including mechanical exhaust or laboratory fume hood. Follow standard laboratory practices.
- **Individual protection measures:**
  Respiratory protection:
  Use appropriate respirator if there is inadequate ventilation by following the government standards.
  Hand protection:
  Wear gloves and use proper glove removal technique to avoid skin contact. Discard gloves after use by following the applicable laboratory regulations. Wash and dry hands.
  Eye/face protection:
  Safety goggles with side-shields approved under appropriate government standards.
  Skin/body protection:
  Use appropriate clothing, footwear and any additional protection measures to protect from splashing or contamination.

Article 9 – Physical and Chemical Properties

Appearance: Colorless fluid.	**Danger of explosion:** Product does not present an explosion hazard.
Odour/Odour Threshold: Not determined.	**Explosion limits:** Lower: 0.9 Vol %; Upper: 0.0 Vol %.
pH: Not available.	**Decomposition temperature:** Not available.
Melting point/freezing point: Not determined.	**Vapor pressure at 20 °C:** 0.1 hPa
Boiling point/Boiling range: 100 °C.	**Density:** Not determined.
Flash point: > 100 °C.	**Relative density:** Not determined.
Flammability (solid, gaseous): Not determined.	**Vapor density:** Not determined.
Ignition temperature: 400 °C.	**Evaporation rate:** Not determined.
Auto-igniting: Product is not self-igniting.	**Solubility in / Miscibility with Water:** Fully miscible.

Article 10 - Stability and Reactivity

- **Reactivity:** Stable under recommended transport and storage conditions.
- **Chemical stability:** Stable under recommended transport and storage conditions.
- **Possible hazardous reactions:** No dangerous reactions known.
- **Conditions to avoid:** Heat and moisture.
- **Incompatible materials:** Strong acids/bases, strong oxidizing/reducing agents.
- **Hazardous decomposition products:** Carbon oxides may formed under fire conditions; no known decomposition information for other decomposition products.

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Article 11 - Toxicological Information

- **Acute toxicity:** Not available.
- **LD/LC50:** Not available.
- **Skin corrosion/irritation:** Not available.
- **Serious eye damage/eye irritation:** Not available.
- **Respiratory or skin sensitization:** Not available.
- **Germ cell mutagenicity:** Not available.
- **Carcinogenicity:** No components are listed in IARC, or NTP, or OSHA, or ACGIH.
- **Reproductive toxicity:** Not available.
- **Teratogenicity:** Not available.
- **Specific target organ toxicity - single exposure/ - repeated exposure (GHS):** Not available.
- **Aspiration hazard:** Not available.
- **Potential health effects:**
  Inhalation: May be harmful if inhaled. May cause respiratory tract irritation.
  Ingestion: May be harmful if swallowed.
  Skin: May be harmful if absorbed through skin. May cause skin irritation.
  Eyes: May cause eye irritation.
- **Signs and Symptoms of Exposure:**
  Prolonged or repeated exposure can cause: Nausea, Dizziness.
- **Synergistic effects:** Not available.

Article 12 - Ecological Information

- **Eco-toxicity:** Not applicable.
- **Biodegradability:** Not applicable.
- **Bio-accumulative potential:** Not applicable.
- **Mobility in soil:** Not applicable.
- **PBT and vPvB assessment:** Not applicable.
- **Other adverse effects:** Not applicable.

Article 13 - Disposal Considerations

- **Disposal methods:** In accordance to applicable national, regional, or local laws and regulations. For additional handling information and protection of employees please refer to Article 7 and 8.
- **Contaminated packaging:** Disposal should be made in accordance to official regulations. Use water or cleansing agents to clean the area.

Article 14 - Transport Information

- **DOT:** Not dangerous goods.
- **IMDG:** Not dangerous goods.
- **IATA:** Not dangerous goods.

Article 15 – Regulatory Information

- **WHMIS Classification:** Non-hazardous.
- **GHS label elements:** Not applicable.
- **Signal word:** Not applicable.
- **Hazard statements:** Not applicable.

Article 16 - Other Information

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.