Catalog #	Aliquot Size
T03-20H-05	5 µg
T03-20H-10	10 µg

PTEN, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog # T03-20H
Lot # N3663-7

Product Description

Recombinant full-length human PTEN was expressed by baculovirus in Sf9 insect cells using an N-terminal His tag. The gene accession number is NM_000314.

Alternative Name(s)

ZS; DEC; GLM2; MHAM; TEP1; MMAC1; PTEN1; 10q23del; MGC11227

Formulation

Recombinant protein stored in 20mM MOPS, pH 7.0, 300mM NaCl, 150mM imidazole, 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.

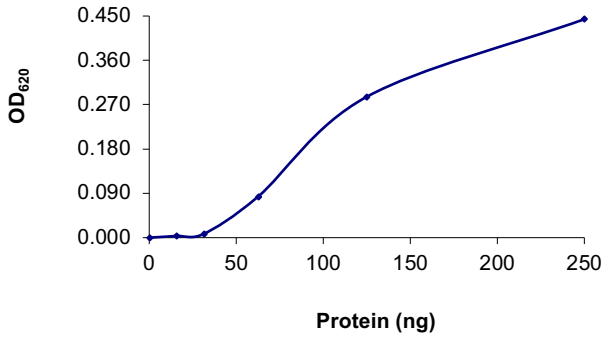
Scientific Background

PTEN (phosphatase and tensin homolog) is a tumor suppressor that is frequently mutated in a large number of cancers (1). PTEN has phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase activity and contains a tensin like domain as well as a catalytic domain similar to that of the dual specificity protein tyrosine phosphatases. Unlike most of the protein tyrosine phosphatases, PTEN preferentially dephosphorylates phosphoinositide substrates and is responsible for negatively regulating the intracellular levels of phosphatidylinositol-3,4,5-trisphosphate in cells. PTEN functions as a tumor suppressor by negatively regulating the PI3K/AKT signaling pathway (2).

References

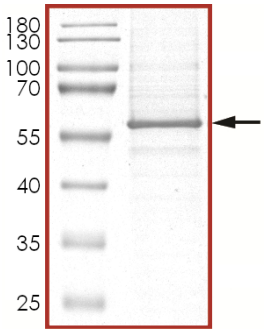
1. Butler, M.G. et al: Subset of individuals with autism spectrum disorders and extreme macrocephaly associated with germline PTEN tumour suppressor gene mutations. *J. Med. Genet. 2005;42(4):318-21.*
2. Steck, P.A. et aal: Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nat. Genet. 1997; 15(4):356-62*

Specific Activity



The specific activity of PTEN was determined to be **505 nmol phosphate released/min/mg** as per activity assay protocol.

Purity



The purity of PTEN was determined to be **>70%** by densitometry. Observed MW **~60 kDa**. Calculated MW **~46 kDa**.

PTEN, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog #	T03-20H
Specific Activity	505 nmol/min/mg
Lot #	N3663-7
Purity	>70%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	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. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Phosphatase (Catalog #: T03-20H)

Active PTEN (0.05μg/μl) diluted with PTEN Dilution Buffer and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active PTEN for optimal results).

PTEN Assay Buffer

PTEN Dilution Buffer contains 50mM TRIS-HCl, pH7.5, -1% (w/v) Octyl β-D-glucopyranoside, add fresh DTT to 1mM prior to use.

Substrate Assay Solution

Phosphatidylinositol (3,4,5) tri-phosphate (PI(3,4,5)P_3) diluted in PTEN Assay Buffer to a working concentration of 400 μM.

Detection Solution

BIOMOL GREEN reagent phosphatase detection kit (BioMol Catalog #: AK-111).

Assay Protocol

Step 1. Prepare a fresh batch of PTEN Assay Buffer and keep on ice.

Step 2. Prepare phosphate standard curve following the instruction of BIOMOL GREEN reagent phosphatase detection kit. Briefly, prepare 1:1 serial dilutions of phosphate standard solutions with PTEN Assay Buffer in a volume of 20 μl. Also, use 20 μl PTEN Dilution Buffer as a blank. The range of phosphate amount should be 0~2 nmol.

Step 3. Thaw the Active PTEN on ice. Prepare serial dilutions of PTEN using PTEN Assay Buffer.

Step 4. In a black full-area 96-well plate with clear bottom, add the following reaction components to bring the total volume to 20 μl:

Component 1. 10 μl of diluted Active PTEN (Catalog #T03-20H)

Component 2. 10 μl of Substrate Assay Solution

Step 5. Set up the blank control as outlined in step 4, excluding the addition of the Active Phosphatase. Replace the Active Phosphatase with an equal volume of PTEN Assay Buffer (Catalog # P22-09).

Step 6. Mix the plate for 1 minute on a tabletop orbital shaker and incubate at 37°C for 45 minutes. Briefly shake the plate at 10 minutes after starting the incubation.

Step 7. Add 100μl BIOMOL GREEN Reagent to each test and control well, as well as the standard curve wells. Do NOT shake.

Step 8. Incubate at room temperature for 30 minutes to allow color development

Step 9. Read plate a spectrophotometer at 620 nm.

Step 10. Plot the free phosphate standard curve. Determine absorbance (y) for each sample (where y = absorbance of sample – background absorbance) and calculate the corresponding nmol phosphate released (x) during the assay using the equation y = A*x + B or x =[y - B] / A (the A and B values are determined from linear-fit equation from the standard curve).

Step 11. Calculate the phosphatase specific activity (SA):

Phosphatase Specific Activity (SA) (nmol/min/mg)

SA = Corresponding phosphate released *1000/ [(Reaction time in min)*(Enzyme amount in μg)]

SAFETY DATA SHEET

Article 1 – Product Identification

Product Name: PTEN, Active **Catalog # T03-20H**

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	≤30%
NaCl	Sodium chloride	7647-14-5	1.753%
Imidazole	1,3-Diaza-2,4-cyclopentadiene	288-32-4	≤1.02%
MOPS	3-(N-morpholino) propanesulfonic acid	1132-61-2	0.418%
Protein		No data available	≤0.02%
DTT; Dithiothreitol	(R*,R*)-1,4-Dimercaptobutane-2,3-diol	3483-12-3	0.0038%
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:** Breath 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.

SAFETY DATA SHEET

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 safe 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/m^3 for mist	British Columbia, Canada
TWA	3 mg/m^3 for respirable mist	British Columbia, Canada
TWA	10 mg/m^3	Alberta, Canada
TWAEV	10 mg/m^3	Ontario, Canada
TWAEV	10 mg/m^3	Quebec, Canada
TWA	10 mg/m^3	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 form under fire conditions; no known decomposition information for other decomposition products.

SAFETY DATA SHEET

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.