



Catalog #	Aliquot Size
P95-31G -05	5 µg
P95-31G -10	10 µg

## PDE7A, Active

Recombinant human protein expressed in Sf9 cells

Catalog # P95-31G

Lot # M333-2

### Product Description

Recombinant human PDE7A (104-end) was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The gene accession number is [NM\\_002603](#).

### Alternative

HCP1; PDE7

### 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.

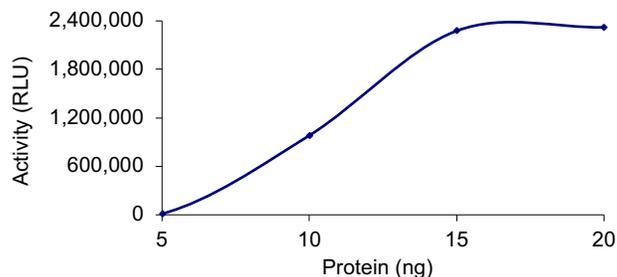
### Scientific Background

PDE7A is a member of the phosphodiesterase family of proteins that play a critical role in regulating intracellular levels of cAMP and cGMP. PDE7A is a high-affinity cAMP-specific PDE and is expressed in T cell lines, peripheral blood T lymphocytes, epithelial cell lines, airway and vascular smooth muscle cells, lung fibroblasts, and eosinophils. PDE7 plays a critical role in the regulation of human T cell function and selective PDE7 inhibitors are being examined to treat immunological and inflammatory disorders (1). PDE7A plays an important role in the regulation of osteoblastic differentiation (2). PDE7A depletion by RNAi upregulates expression of several osteogenic genes and increases mineralization.

### References

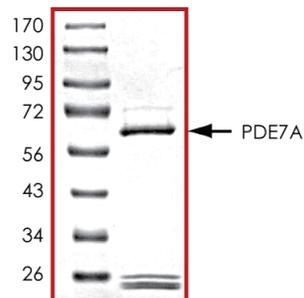
1. Nakata A, et al: Potential role of phosphodiesterase 7 in human T cell function: comparative effects of two phosphodiesterase inhibitors. Clin Exp Immunol. 2002 Jun;128(3):460-6.
2. Pekkinen M, et al: Effects of phosphodiesterase 7 inhibition by RNA interference on the gene expression and differentiation of human mesenchymal stem cell-derived osteoblasts. Bone. 2008 Jul;43(1):84-91.

### Specific Activity



The specific activity of PDE7A was determined to be **166 nmol/min/mg** as per activity assay protocol.

### Purity



The purity of PDE7A was determined to be **>85%** by densitometry. Observed MW **~64 kDa**. Calculated MW **~64 kDa**.

## PDE7A, Active

Recombinant human protein expressed in Sf9 cells

Catalog #	P95-31G
Specific Activity	166 nmol/min/mg
Lot #	M333-2
Purity	>85%
Concentration	0.1 µg/µl
Stability	1 yr 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 PDE7A (Catalog #: P95-31G)

Active PDE7A (0.1µg/µl) diluted with 1X PDE-Glo™ Reaction 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 PDE7A for optimal results).

### 100 mM IBMX Solution

Prepare 100 mM of 3-isobutyl-1-methylxanthine (IBMX) in 100% DMSO. Store aliquots at -20°C.

### PDE-Glo™ Phosphodiesterase Assay Kit (Promega, Cat # V1361)

cAMP and cGMP solution, 1 mM  
PDE-Glo™ Reaction Buffer, 5X  
PDE-Glo™ Termination Buffer, 5X  
PDE-Glo™ Detection Buffer, 5X  
Protein Kinase A (PKA)  
Kinase-Glo™ Substrate  
Kinase-Glo™ Buffer

## Assay Protocol

The PDE7A assay is performed using the PDE-Glo™ Phosphodiesterase Assay kit (Promega; Cat# V1361). The assay involves first a PDE7A reaction between an active PDE7A preparation and a cyclic nucleotide substrate (cAMP). Then PDE-Glo™ Termination Buffer and PDE-Glo™ Detection Buffer (which contains ATP, inactive PKA and PKA substrate) are added to the reaction. The cyclic nucleotide substrate remaining after the PDE7A reaction can bind to the inactive PKA regulatory subunit thereby releasing the active catalytic subunit of PKA. The active catalytic subunit of PKA then catalyzes phosphorylation of the PKA substrate in the presence of ATP which leads to a reduction in ATP level. In the final step, Kinase-Glo™ reagent is added to measure the Luciferase activity towards Luciferin and the luminescent signal produced is related to the amount of ATP remaining which is indirectly related to the activity of PDE7A.

**Step 1.** Thaw the Active PDE7A and PDE-Glo™ Phosphodiesterase Assay Kit reagents on ice.

**Step 2.** Prepare the following working solutions:

- o Diluted active PDE7A with 1X PDE-Glo™ Reaction Buffer on ice
- o 2µM cAMP substrate solution in 1X PDE-Glo™ Reaction Buffer at ambient temperature
- o 1X PDE-Glo™ Termination Buffer in 10 mM IBMX solution at ambient temperature
- o 1X PDE-Glo™ detection solution (mix 8µl PKA with 792µl water and 200µl 5X PDE-Glo™ Detection Buffer). Prepare immediately before use
- o Kinase-Glo™ reagent by adding Kinase-Glo™ Buffer to Kinase-Glo™ Substrate at ambient temperature

**Step 3.** In a polystyrene 96-well plate, add the following components bringing the initial reaction volume up to 25µl:

**Component 1.** 12.5µl of diluted Active PDE7A (Catalog #P95-31G)

**Component 2.** 12.5µl of 2µM cAMP solution (0.025 nmol cAMP used per assay)

**Step 4.** Set up a blank control as outlined in step 3 by excluding the addition of the diluted PDE preparation. Replace the PDE preparation with an equal volume of 1X PDE-Glo™ Reaction Buffer.

**Step 5.** Initiate the reaction by adding cAMP substrate solution and incubate the mixture at 30°C for 10 minutes on a plate shaker.

**Step 6.** Terminate the PDE reaction by adding 12.5µl of 1X PDE-Glo™ Termination Buffer. Mix well.

**Step 7.** Add 12.5µl of 1X PDE-Glo™ detection solution. Mix well and then incubate at ambient temperature for 20 minutes.

**Step 8.** After the incubation period, add 50µl of Kinase-Glo™ reagent mix and then incubate at ambient temperature for 10 min.

**Step 9.** Read the polystyrene 96-well reaction plate using the KinaseGlo Luminescence Protocol on a GloMax plate reader (Promega; Cat# E7031).

**Step 10.** Perform a cAMP standard curve. Determine RLU at each concentration. Then calculate the corresponding nmol cAMP remaining after the PDE reaction from the standard curve.

**Step 11.** Calculate the PDE specific activity as outlined below.

### PDE Specific Activity (SA) (nmol/min/mg)

$$[\text{cAMP total (nmol)} - \text{cAMP remaining (nmol)}] / (\text{Reaction time in min}) * (\text{Enzyme amount in mg})$$

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## Article 1 – Product Identification

**Product Name: PDE7A, Active**

**Catalog # P95-31G**

*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	0.0038%
EDTA	Ethylenediaminetetraacetic acid	6381-92-6	0.0037%
PMSF; Phenylmethanesulfonyl fluoride	α-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 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 <sup>3</sup> for mist	British Columbia, Canada
TWA	3 mg/m <sup>3</sup> for respirable mist	British Columbia, Canada
TWA	10 mg/m <sup>3</sup>	Alberta, Canada
TWAEV	10 mg/m <sup>3</sup>	Ontario, Canada
TWAEV	10 mg/m <sup>3</sup>	Quebec, Canada
TWA	10 mg/m <sup>3</sup>	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

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

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- **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.

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## Article 12 - Ecological Information

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- **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.

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## Article 13 - Disposal Considerations

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- **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.

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## Article 14 - Transport Information

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

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## Article 15 - Regulatory Information

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

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## Article 16 - Other Information

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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.

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