

#### DESCRIPTION

<b>Source</b>	Chinese Hamster Ovary cell line, CHO-derived human PSMA/FOLH1/NAALADase I protein			
	Avi-tag	MD	Human IgG1 (Pro100-Lys330)	IEGR
	N-terminus		Human NAALADase-1 (Lys44-Ala750) Accession # Q04609.1	
	C-terminus			
<b>N-terminal Sequence Analysis</b>	Gly			
<b>Structure / Form</b>	Biotinylated via Avi-tag			
<b>Predicted Molecular Mass</b>	108 kDa			

#### SPECIFICATIONS

<b>SDS-PAGE</b>	112-125 kDa, under reducing conditions.
<b>Activity</b>	Measured by its ability to hydrolyze the substrate N-acetyl-L-Asp-L-Glu into N-acetyl-L-Asp and L-Glu. The L-Glu product is measured by fluorescence after its derivatization by <i>ortho</i> -phthalaldehyde. The specific activity is >325 pmol/min/μg, as measured under the described conditions.  Measured by its binding ability in a functional ELISA. Biotinylated Recombinant Human PSMA/FOLH1/NAALADase I Avi-tag Fc Chimera (Catalog # AV111535) binds Human PSMA/FOLH1/NAALADase I Antibody (Catalog # MAB4234) with an ED <sub>50</sub> of 0.0750-1.20 μg/mL.
<b>Endotoxin Level</b>	<0.10 EU per 1 μg of the protein by the LAL method.
<b>Purity</b>	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
<b>Formulation</b>	Supplied as a 0.2 μm filtered solution in MES and NaCl. See Certificate of Analysis for details.

#### Activity Assay Protocol

<b>Materials</b>	<ul style="list-style-type: none"> <li>Assay Buffer: 50 mM HEPES, 100 mM NaCl, pH 7.5</li> <li>o-PA Buffer: 0.2 M NaOH containing 0.1% β-Mercaptoethanol (v/v)</li> <li>Recombinant Human PSMA/FOLH1/NAALADase-1/N- Avitag/Fc (rhPSMA) (Catalog # AV111535)</li> <li>Substrate: Ac-Asp-Glu, 10 mM stock in 40 mM NaOH</li> <li>o-phthalaldehyde (o-PA), 50 mg/mL (373 mM) stock in DMSO</li> <li>Black 96-well Plate</li> <li>Plate Reader with Fluorescence Read Capability</li> </ul>
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<b>Assay</b>	<ol style="list-style-type: none"> <li>Dilute rhPSMA to 0.4 μg/mL in Assay Buffer.</li> <li>Dilute Substrate to 40 μM in Assay Buffer.</li> <li>Combine 125 μL of 0.4 μg/mL rhPSMA and 125 μL of 40 μM Substrate. For a control, inactivate 125 μL of 0.4 μg/mL rhPSMA by heating it at 95 °C for 5 minutes, then add 125 μL of 40 μM Substrate.</li> <li>Incubate reactions and controls at 37 °C for 60 minutes.</li> <li>Stop the reaction by heating reactions and controls at 95 °C for 5 minutes, then cool to room temperature.</li> <li>Prepare a 15 mM o-PA solution in o-PA Buffer.</li> <li>Add 250 μL of 15 mM o-PA solution to each reaction and control. Vortex and incubate at room temperature for 10 minutes.</li> <li>Load 200 μL of reactions and controls to plate.</li> <li>Read at excitation and emission wavelengths of 330 nm and 450 nm (top read), respectively, in endpoint mode.</li> <li>Calculate specific activity:</li> </ol>
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$$\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\text{Adjusted Fluorescence* (RFU)} \times \text{Conversion Factor** (pmol/RFU)}}{\text{Incubation time (min)} \times \text{amount of enzyme (}\mu\text{g)}}$$

\*Adjusted for Control

\*\*Derived using calibration standard L-Glutamic Acid

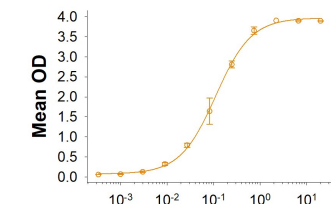
<b>Final Assay Conditions</b>	Per Well: <ul style="list-style-type: none"> <li>rhPSMA: 0.02 μg</li> <li>Substrate: 10 μM</li> <li>o-PA: 7.5 mM</li> </ul>
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#### PREPARATION AND STORAGE

<b>Shipping</b>	The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.
<b>Stability &amp; Storage</b>	<b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"> <li>6 months from date of receipt, -20 to -70 °C as supplied.</li> <li>3 months, -20 to -70 °C under sterile conditions after opening.</li> </ul>

**DATA**

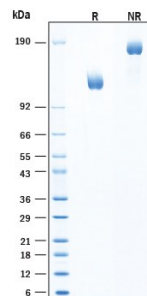
**Binding Activity**



**Biotinylated Recombinant Human PSMA/FOLH1/NAALADase I Avi-tag (µg/mL)**

**Biotinylated Recombinant Human PSMA/FOLH1/NAALADase I Avi-tag Fc Chimera Protein Binding Activity.** Biotinylated Recombinant Human PSMA/FOLH1/NAALADase I Avi-tag Fc Chimera Protein (Catalog # AV111535) binds Human PSMA/FOLH1/NAALADase I Antibody (Catalog # MAB4234) with an ED<sub>50</sub> of 0.0750-1.20 µg/mL.

**SDS-PAGE**



**Biotinylated Recombinant Human PSMA/FOLH1/NAALADase I Avi-tag Fc Chimera Protein SDS-PAGE.** 2 µg/lane of Biotinylated Recombinant Human PSMA/FOLH1/NAALADase I Avi-tag Fc Chimera Protein (Catalog # AV111535) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 112-125 kDa, under reducing conditions.

**BACKGROUND**

Prostate-specific membrane antigen (PSMA), a tumor marker in prostate cancer encoded by the FOLH1 gene, is a type II transmembrane zinc metallopeptidase that is most highly expressed in the nervous system, prostate, kidney, and small intestine (1,2). PSMA has a short cytosolic N-terminal domain, a single membrane-spanning segment, and an extracellular region that is composed of a protease domain, apical domain, and C-terminal domain (3). The extracellular domains all contribute to substrate recognition. The protein forms an active homodimer reliant on interactions between amino-acid side chains and glycosylation (3,4). PSMA is also known as glutamate carboxypeptidase II (GCP II), folate hydrolase 1, and N-acetylated-alpha-linked acidic dipeptidase-1 (NAALADase1). PSMA activity plays a role in tumor angiogenesis making it not only a tumor marker, but a therapeutic target in cancers including prostate cancer (5). In the brain, PSMA hydrolyzes the neurotransmitter N-acetyl-Asp-Glu (NAAG) to produce glutamate, another neurotransmitter. Inhibition of brain PSMA activity is considered to be a promising approach for the treatment of neurological disorders associated with glutamate excitotoxicity such as stroke, schizophrenia, Alzheimer's, and amyotrophic lateral sclerosis (5,6,7). Intestinal PSMA hydrolyzes folylpoly-γ-glutamates, facilitating the uptake of folate (8). Upregulation of PSMA is present in inflammatory bowel disease, Crohn's disease, and ulcerative colitis where pharmacological inhibition has shown amelioration of clinical symptoms pertaining to these diseases in mice (5).

**References:**

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