Biotinylated Recombinant Human PSMA/FOLH1/NAALADase I

Catalog Number: BT4234

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human PSMA/FOLH1/NAALADase I protein Lys44-Ala750 with an N-terminal 6-His tag Accession # NP_004467.1
N-terminal Sequence Analysis	His
Structure / Form	Biotinylated via amines.
Predicted Molecular Mass	80 kDa

SPECIFICATIONS

SDS-PAGE	94-104 kDa, under reducing conditions
Activity	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 *ortho*-phthaldialdehyde. The specific activity is >250 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 (Catalog # BT4234) binds Human PSMA/FOLH1/NAALADase I Antibody (Catalog # MAB4234) with an ED_{50} of 50.0-600 ng/mL.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.
Formulation	Supplied as a 0.2 µm filtered solution in MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM HEPES, 100 mM NaCl, pH 7.5
- OPA Buffer: 0.2 M NaOH containing 0.1% β-Mercaptoethanol (v/v)
- Biotinylated Recombinant Human PSMA/FOLH1/NAALADase1 (rhPSMA) (Catalog # BT4234)
- Substrate: Ac-Asp-Glu, 10 mM stock in 40 mM NaOH
- ortho-phthaldialdehyde (OPA), 50 mg/mL (373 mM) stock in DMSO
- Black 96 well Plate
- Plate Reader with Fluorescence Read Capability

Assay

1. Dilute rhPSMA to 0.4 µg/mL in Assay Buffer.
2. Dilute Substrate to 40 µM in Assay Buffer.
3. Combine 125 µL of 0.4 µg/mL rhPSMA and 125 µL of 40 µM Substrate. For 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.
4. Incubate reactions and controls at 37 °C for 1 hour.
5. Stop the reactions and controls by heating at 95 °C for 5 minutes, then cool to room temperature.
6. Prepare a 15 mM OPA solution in OPA Buffer.
7. Add 250 µL of OPA solution to all vials and vortex.
8. Incubate at room temperature for 10 minutes.
9. Load 200 µL of reactions and controls to plate.
10. Read at excitation and emission wavelengths of 330 nm and 450 nm (top read), respectively, in endpoint mode.
11. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Fluorescence* (RFU) x Conversion Factor** (pmol/RFU)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Adjusted for Control
**Derived using calibration standard L-Glutamic Acid.

Final Assay Conditions

Per Well:

- rhPSMA: 0.020 µg
- Substrate: 10 µM
- OPA: 7.5 mM

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.
Stability & Storage	**Use a manual defrost freezer and avoid repeated freeze-thaw cycles.** • 6 months from date of receipt, -70 °C as supplied. • 3 months, -70 °C under sterile conditions after opening.

DATA

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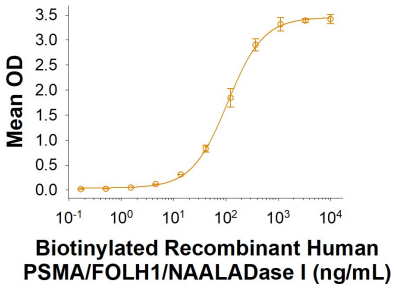
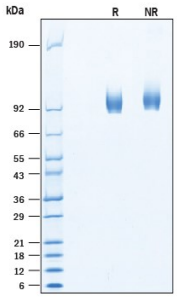
Binding Activity



Biotinylated Recombinant Human PSMA/FOLH1/NAALADase I Protein Binding Activity. Biotinylated Recombinant Human PSMA/FOLH1/NAALADase I Protein (Catalog # BT4234) binds Human PSMA/FOLH1/NAALADase I Antibody (Catalog # MAB4234) with an ED_{50} of 50.0-600 ng/mL.

SDS-PAGE



Biotinylated Recombinant Human PSMA/FOLH1/NAALADase I Protein SDS-PAGE. 2 µg/lane of Biotinylated Recombinant Human PSMA/FOLH1/NAALADase I Protein (Catalog # BT4234) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 94-104 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). PMSA 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 (GCPII), 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 (6,7,8). 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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