

**DESCRIPTION**

**Source** Human embryonic kidney cell, HEK293-derived human Fatty Acid Synthase/FASN protein  
Met1-Gly2511, with a C-terminal 6-His tag  
Accession # P49327.3

**N-terminal Sequence Analysis** Protein identity confirmed by mass spectrometry.

**Predicted Molecular Mass** 274 kDa

**SPECIFICATIONS**

**Activity** Measured by its ability to hydrolyze 4-methylumbelliferyl heptanoate.

**Endotoxin Level** <0.10 EU per 1 µg of the protein by the LAL method.

**Purity** >90%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

**Formulation** Supplied as a 0.2 µm filtered solution in Tris, NaCl, Glycerol and TCEP. See Certificate of Analysis for details.

**Activity Assay Protocol**

- Materials**
- Assay Buffer: 50 mM Tris, 100 mM NaCl, 0.02% Brij-35 (w/v), pH 7.5
  - Reading Buffer: 0.1 M Tris, pH 9.0
  - Recombinant Human Fatty Acid Synthase/FASN His-tag (rhFASN) (Catalog # 11788-FS)
  - Substrate: 4-methylumbelliferyl heptanoate, 10 mM stock in DMSO
  - Black 96-well Plate
  - Plate Reader with Fluorescent Read Capability

- Assay**
1. Dilute rhFASN to 100 µg/mL in Assay Buffer.
  2. Dilute Substrate to 200 µM in Assay Buffer.
  3. Combine equal volumes of 100 µg/mL rhFASN and 200 µM Substrate. Include a Substrate Blank containing equal volumes of Assay Buffer and 200 µM Substrate.
  4. Incubate reactions and Substrate Blank at room temperature for 5 minutes. Protect from light.
  5. Load 10 µL of each reaction and blank into wells of a black plate.
  6. Add 90 µL of Reading Buffer to each well.
  7. Immediately read plate at excitation and emission wavelengths of 365 nm and 445 nm, respectively, in endpoint mode.
  8. Calculate specific activity:

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

\*Adjusted for Substrate Blank

\*\*Derived from calibration standard 4-methylumbelliferone.

- Final Assay Conditions**
- Per Reaction:
- rhFASN: 50 µg/mL
  - Substrate: 100 µM

**PREPARATION AND STORAGE**

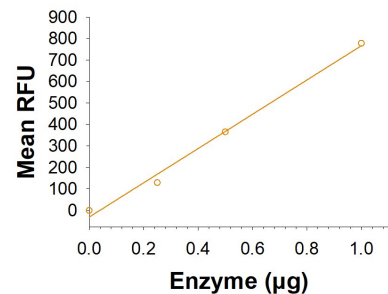
**Shipping** The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

**Stability & Storage** Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

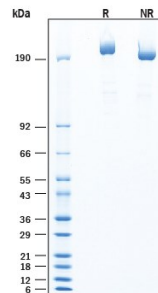
**DATA**

**Enzyme Activity**



**Recombinant Human Fatty Acid Synthase/FASN His-tag Enzyme Activity.** Recombinant Human Fatty Acid Synthase/FASN His-tag (Catalog # 11788-FS) is measured by its ability to hydrolyze 4-methylumbelliferyl heptanoate.

**SDS-PAGE**



**Recombinant Human Fatty Acid Synthase/FASN His-tag SDS-PAGE.** 2 µg/lane of Recombinant Human Fatty Acid Synthase/FASN His-tag (Catalog # 11788-FS) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 234-258 kDa, under reducing conditions.

**BACKGROUND**

Recombinant human fatty acid synthase (FASN) is a large cytosolic, homodimeric, multifunctional protein that functionally completes the entire pathway of palmitate synthesis from malonyl-CoA in mammals. Each FASN monomer contains three N-terminal domains, the b-ketoacyl synthase (KS), malonyl/acetyl transferase (MAT) and dehydratase (DH) separated by a structural core from four C-terminal domains, the enoyl reductase (ER), b-ketoacyl reductase (KR), acyl carrier protein (ACP) and thioesterase (TE) (1-2). The highly coordinated assembly of these domains in a homodimeric structure allows FASN to catalyze the sequential condensation of acetyl-CoA and malonyl-CoA, utilizing NADPH to produce palmitate as the final product (3). In contrast to normal adult tissues, where exogenous dietary lipids are generally sufficient to support metabolic demands, tumor cells display a marked dependency on fatty acid (FA) synthesis (3-5). FASN has two key dynamic post-translational modifications that impact its stability, subcellular localization, and enzymatic activity: S-palmitoylation that results in protein stabilization and elevated expression (6-7) and acetylation that prevents its degradation via the ubiquitin-mediated proteasomal degradation pathway (8). FASN is broadly expressed but overexpression and hyperactivation of FASN is reported in solid and hematologic malignancies, including but not limited to breast, liver, prostate, cervical, and lymphoid cancers (6,7,9-13), as elevated FASN increases the biosynthesis of membrane phospholipids and lipid rafts that facilitate sustained oncogenic signaling and its downstream lipid products support enhanced migratory and metastatic behavior (3). FASN expression is prominently regulated by sterol regulatory element-binding protein 1 (SREBP1) (13) and tumor-suppressive miRNAs that target FASN, such as miR-497-5p or miR-449 (11). Through its roles in metabolic adaptability and modulation of immune checkpoint inhibition through MHC-I downregulation, CD8<sup>+</sup> T-cell exhaustion, and induction of tumor-associated macrophage M2 polarization, FASN is implicated in resistance to chemotherapy, radiotherapy, and emerging therapies in cancer (3,7,12,14). Therapeutic approaches to modulate FASN initially focused on direct enzymatic inhibitors that target the TE domain (such as cerulenin, C75, and orlistat) with some success (9) while subsequent KS-domain inhibitors (such as denifanstat and GSK2194069) had potent, on-target suppression and broad antitumor activity in preclinical models (2). Next-wave inhibitors seek to exploit tumor-specific enzymatic activation (15). In addition, strategies for indirect modulation via targeted perturbation of upstream or post-translational regulators of FASN are actively being explored (16-17) as well as rational combinatorial strategies that yield more durable anti-tumor responses based on biomarker-guided deployment (3, 14, 18).

**References:**

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