

Background

NMNAT-1 is expressed in the nuclei of all human tissues, with highest expression in skeletal muscle, heart, kidney, pancreas, and brain (1). The enzyme transfers adenylate from ATP to nicotinamide ribonucleotide or nicotinate ribonucleotide to generate NAD⁺ or deamido-NAD⁺, and is an essential enzyme for the production of nuclear NAD⁺ (2). Nuclear NAD⁺ is required by poly(ADP-ribose) polymerase 1 (PARP-1), which poly-ADP-ribosylates chromatin in response to DNA strand breaks. NMNAT-1 is known to interact with PARP-1, resulting in its activation, but this interaction with PARP-1 is prevented when NMNAT-1 is phosphorylated at Ser136 (3). Nuclear NAD⁺ levels are also important for the regulation of SIR2 histone deacetylases (4). A naturally occurring Ube4b/NMNAT-1 chimeric protein is directly involved in slowing the degeneration of injured neurons in mice (5). NMNAT activity is required for the activation of tiazofurin, a drug used to treat leukemia (6). Two other NMNAT enzymes are present in humans. NMNAT-2 is localized in the Golgi complex and cytoplasm, and NMNAT-3 is a mitochondrial enzyme (7).

References:

1. Emanuelli, M. *et al.* (2001) *J. Biol. Chem.* **276**:406.
2. Schweiger, M. *et al.* (2001) *FEBS Lett.* **492**:95.
3. Berger, F. *et al.* (2007) *Proc. Natl. Acad. Sci. USA* **104**:3765.
4. Revollo, J.R. *et al.* (2004) *J. Biol. Chem.* **279**:50754.
5. Mack, T.G. *et al.* (2001) *Nature Neurosci.* **4**:1199.
6. Boulton, S. *et al.* (1997) *Br. J. Cancer* **76**:845.
7. Berger, F. *et al.* (2005) *J. Biol. Chem.* **280**:36334.

Description

Source	<i>Spodoptera frugiperda</i> , Sf21 (baculovirus)-derived Met1 - Thr279, with a C-terminal 6-His tag. Accession # Q9HAN9
N-terminal Sequence Analysis	Ser4
Structure / Form	Non-covalently linked hexamer
Predicted Molecular Mass	32 kDa

Specifications

SDS-PAGE	33 kDa, reducing conditions
Activity	Measured by the production of NAD ⁺ , which is converted to NADH by alcohol dehydrogenase. The specific activity is > 2,500 pmoles/min/μg, as measured under the described conditions. See Activity Assay Protocol.
Endotoxin Level	<1.0 EU per 1 μg of the protein by the LAL method.
Purity	>90%, by SDS-PAGE under reducing conditions and visualized by silver stain.
Formulation	Supplied as a 0.2 μm filtered solution in Tris, NaCl, Glycerol and DTT. See Certificate of Analysis for details.

Preparation and Storage

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

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM HEPES, pH 7.5
- Recombinant human NMNAT-1 (R&D Systems, Catalog # 5865-NT)
- β-Nicotinamide mononucleotide (β-NMN) (Sigma, Catalog # N3501), 50 mM stock in deionized water
- Adenosine triphosphate (ATP) (Sigma, Catalog # A7699), 10 mM stock in deionized water
- Yeast Alcohol Dehydrogenase (ADH) (Sigma, Catalog # A3263), 5 mg/mL stock in 25 mM MES, 20% Glycerol, pH 6.5
- 1 M Magnesium Chloride
- 95 - 100% Ethanol
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhNMNAT-1 to 0.5 ng/μL in Assay Buffer.
2. Prepare Substrate Mixture: 30 mM MgCl₂, 1 mM β-NMN, 4 mM ATP, 0.1 mg/mL ADH, and 2% Ethanol in Assay Buffer.
3. In a plate, load 50 μL of 0.5 ng/μL rhNMNAT-1, and start the reaction by adding 50 μL of Substrate Mixture to wells. Include a Substrate Blank containing 50 μL Assay Buffer and 50 μL of Substrate Mixture.
4. Read absorbance at a wavelength of 339 nm (bottom read) in kinetic mode for 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmoles/min/}\mu\text{g)} = \frac{\text{Adjusted } V_{\text{max}}^* (\text{OD/min}) \times \text{well volume (L)} \times 10^{12} \text{ pmol/mol}}{\text{ext. coeff}^{**} (\text{M}^{-1}\text{cm}^{-1}) \times \text{path corr.}^{***} (\text{cm}) \times \text{amount of enzyme } (\mu\text{g})}$$

*Adjusted for Substrate Blank

**Using the extinction coefficient 6220 M⁻¹cm⁻¹

***Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD

Final Assay Conditions Per well

- rhNMNAT-1: 0.025 μg
- Substrate Mixture: 0.5 mM β-NMN, 2 mM ATP, 15 mM MgCl₂, 0.05 mg/mL ADH, and 1% Ethanol

4/28/2010

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NOT FOR USE IN HUMANS