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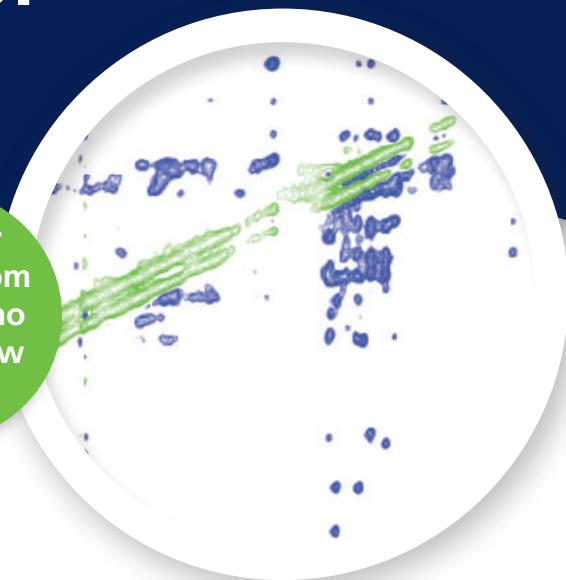
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RESEARCH PRODUCTS

# New Protein Standards!

## Stable Isotope-Labeled Standards and XF-1 Protein Pairs for X-Filtered NOESY

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Cambridge Isotope Laboratories, Inc. (CIL) is pleased to offer new and exciting isotope-enriched proteins for use as standards in NMR spectroscopy. Isotope-enriched protein standards are ideal for:

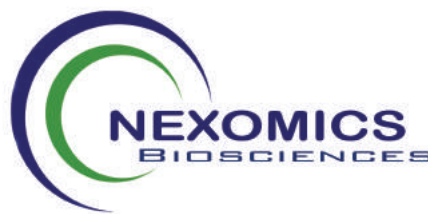
- Aiding in the development of new pulse sequences
- Optimizing parameters for a given pulse sequence
- Assessing spectrometer performance
- Training purposes

Nexomics Biosciences, Inc. is a New Jersey-based contract research organization that specializes in a broad array of gene-to-structure services to the biopharmaceutical community. Gaetano Montelione, CEO of Nexomics Biosciences, Inc., is an expert in the determination of protein structures using NMR. He is a Distinguished Professor of Molecular Biology and Biochemistry at the Center for Advanced Biotechnology and Medicine, Rutgers University, and has over 300 publications.

Nexomics provides high-quality, high-purity standards that are invaluable tools for bioNMR. Each product is accompanied by the following data:

- $^1\text{H}$ - $^{15}\text{N}$  HSQC ( $^{15}\text{N}$ -labeled proteins)
- $^1\text{H}$ - $^{13}\text{C}$  HSQC ( $^{13}\text{C}$ -labeled proteins)
- CO-NH projection of 3D HNCO ( $^{15}\text{N}$ ,  $^{13}\text{C}$ -labeled proteins)
- SDS PAGE (for all labeled proteins)
- MALDI-TOF (for all labeled proteins)
- $^{15}\text{N}$ -edited X-filtered 2D NOESY (NEX-XF1)

**NEW!**  
"ILV" and "ILVfy"  
labeled protein  
standards



## Maltose Binding Protein (NEX-MBP)

NEX-MBP is a 44.9 kDa monomeric protein for which multiple sets of resonance assignments (BMRB database) and 3D structures (PDB database) are publicly available. This product is uniformly  $^2\text{H}$ ,  $^{15}\text{N}$ ,  $^{13}\text{C}$ -enriched with selective incorporation of protons into methyl groups of Ile- $\delta$ 1, Leu- $\delta$  and Val- $\gamma$  side chains. As nonuniform sampling (NUS) and other NMR techniques emerge to push the size limitations of NMR to new boundaries, large protein standards, such as NEX-MBP, will be required to test data-collection and processing strategies.

### NEX-MBP sample formulations:

#### NEX-MBP1: Apo Conformation

0.5 mM  $^2\text{H}$ ,  $^{15}\text{N}$ ,  $^{13}\text{C}$  and ILV methyl  $^1\text{H}$ ,  $^{13}\text{C}$  MBP in 10%  $\text{D}_2\text{O}$ , 0.02%  $\text{NaN}_3$ , 20 mM sodium phosphate @ pH 7.2

#### NEX-MBP2: Closed Conformation

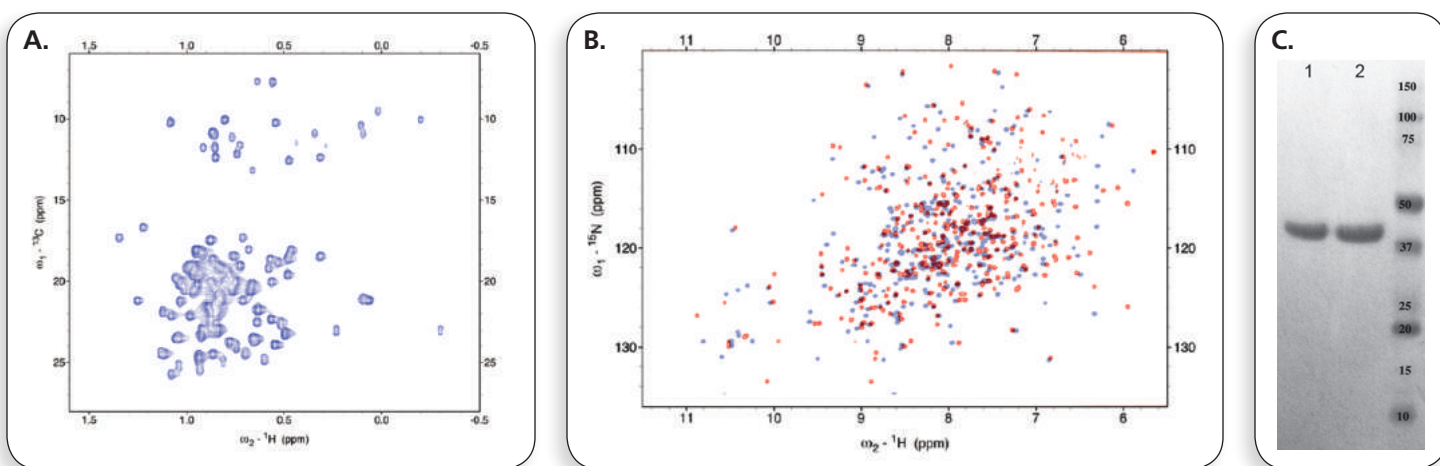
0.5 mM  $^2\text{H}$ ,  $^{15}\text{N}$ ,  $^{13}\text{C}$  and ILV methyl  $^1\text{H}$ ,  $^{13}\text{C}$  MBP with 3 mM maltotriose, 10%  $\text{D}_2\text{O}$ , 0.02%  $\text{NaN}_3$ , 20 mM sodium phosphate @ pH 7.2

#### NEX-MBP3: Open Conformation

0.5 mM  $^2\text{H}$ ,  $^{15}\text{N}$ ,  $^{13}\text{C}$  and ILV methyl  $^1\text{H}$ ,  $^{13}\text{C}$  MBP with 2 mM  $\beta$ -cyclodextrin, 10%  $\text{D}_2\text{O}$ , 0.02%  $\text{NaN}_3$ , 20 mM sodium phosphate @ pH 7.2

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### *E. coli* Maltose Binding Protein (27-396), Apo Conformation

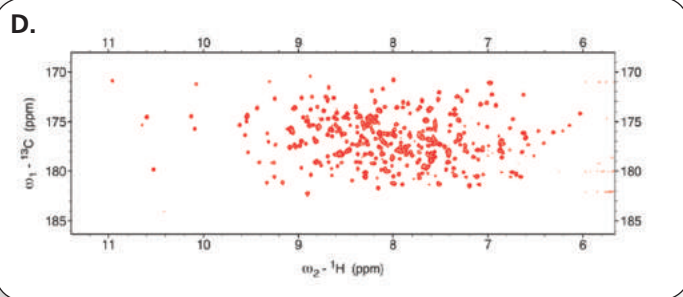
Catalog No.	Label
NEX-MBP1-U-0	unlabeled
NEX-MBP1-N-0	( $^{15}\text{N}$ , 95%)
NEX-MBP1-CN-5-0	( $^{13}\text{C}$ , 5%; $^{15}\text{N}$ , 95%)
NEX-MBP1-CN-0	( $^{13}\text{C}$ , 95%; $^{15}\text{N}$ , 95%)
NEX-MBP1-CDN-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%)
NEX-MBP1-ILV-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILV)
NEX-MBP1-ILV-FY-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILV-FY)

### *E. coli* Maltose Binding Protein (27-396), Closed Conformation

NEX-MBP2-U-0	unlabeled
NEX-MBP2-N-0	( $^{15}\text{N}$ , 95%)
NEX-MBP2-CN-5-0	( $^{13}\text{C}$ , 5%; $^{15}\text{N}$ , 95%)
NEX-MBP2-CN-0	( $^{13}\text{C}$ , 95%; $^{15}\text{N}$ , 95%)
NEX-MBP2-CDN-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%)
NEX-MBP2-ILV-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILV)
NEX-MBP2-ILV-FY-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILV-FY)

### *E. coli* Maltose Binding Protein (27-396), Open Conformation

NEX-MBP3-U-0	unlabeled
NEX-MBP3-N-0	( $^{15}\text{N}$ , 95%)
NEX-MBP3-CN-5-0	( $^{13}\text{C}$ , 5%; $^{15}\text{N}$ , 95%)
NEX-MBP3-CN-0	( $^{13}\text{C}$ , 95%; $^{15}\text{N}$ , 95%)
NEX-MBP3-CDN-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%)
NEX-MBP3-ILV-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILV)
NEX-MBP3-ILV-FY-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILV-FY)



- A.**  $^{13}\text{C}$ ,  $^1\text{H}$  HSQC NEX-MBP3 "open" conformation  
**B.** "Open" (blue) and "closed" (red) superposition  
**C.** SDS-PAGE GEL NEX-MBP  
 NEX-MBP3  $\beta$ -cyclodextrin complexed "open" sample (lane 1)  
 NEX-MBP2 maltotriose complexed "closed" sample (lane 2)  
**D.** CO-NH 2D plane of HNCO triple-resonance experiment of NEX-MBP2 "closed" sample

### Protein Sequence

MKIEEGKLIWINGDKGYNGLAEVGGKFKEDTGKIVTVEHPDKLEEFQVAATGDGPDIIFWAH  
 DRFGGYAQSGLLAETPDKAFQDKLYPFTWDVAVRYNGKLIAYPIAVEALSIIYKDLLNPPKTWEE  
 IPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENKGYDIKDVGVNAGAKGLTFL  
 VDLIKNHMNAADTYSIAEAFNKGGETAMTINGPWAWNSNIDTSKVNYYGVTVLPTFKGQPSKP  
 FVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMEN  
 AQKGEIMPNIQMSAFWYAVRTAVINAASGRQTVDEALKDAQTRITK

## X-Filtered NOESY NMR Standard (NEX-XF1)

In an X-filtered experiment, only NOEs between  $^{15}\text{N}/^{13}\text{C}$ - $^1\text{H}$  and  $^{14}\text{N}/^{12}\text{C}$ - $^1\text{H}$  (e.g. interchain NOEs) protons are observed. NOEs between protons connected to  $^{15}\text{N}$ ,  $^{13}\text{C}$  are filtered (intrachain NOEs). When uniformly double-labeled protein sample is mixed with a natural-abundance protein sample, the interface will give rise to the only observable NOESY cross peaks. This powerful strategy enables the spectroscopist to discern intra from inter NOESY cross peaks, thereby providing essential distance constraints for defining the dimer interface (Lee, et al., 1994, 350:87; Palmer, et al., 1991, 93:151; Schleucher, et al., 1994, 4:301).

NEX-XF1 is a 16 kDa protein (*A. fulgidus* antitoxin vapB21 homodimer) for which a set of resonance assignments (bmr7362), 3D structure (2NWT) and other NMR data are available in the public domain. This is a mixture of unlabeled and uniformly  $^{15}\text{N}$ ,  $^{13}\text{C}$ -enriched protein (25% homodimer unlabeled; 50% heterodimer unlabeled/labeled; 25% homodimer labeled) and is perfect to set up X-filtered NOESY experiments.

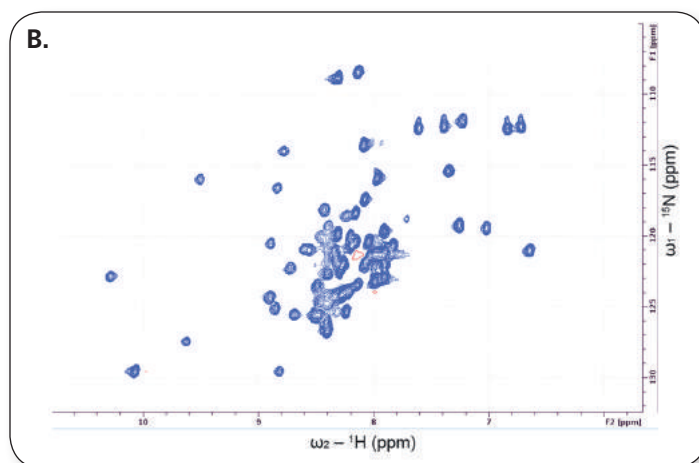
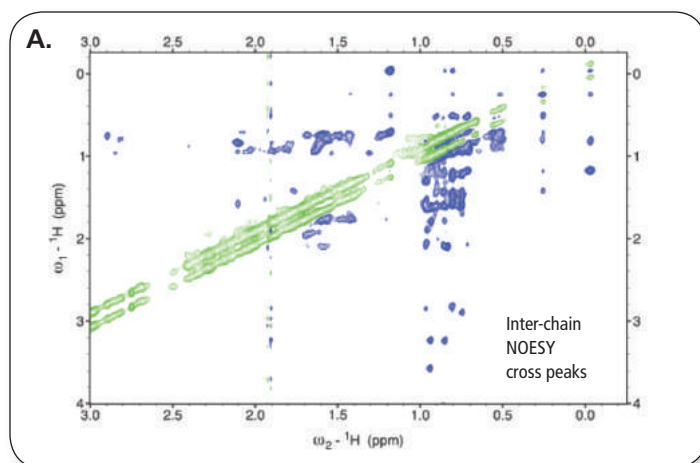
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### NEX-XF1 homodimer sample formulation:

**NEX-XF1:**  $^{13}\text{C}$ ,  $^{15}\text{N}$ -labeled and unlabeled sample conditions

1 mM protein, 20 mM  $\text{NH}_4\text{OAc}$  pH 5.5, 100 mM NaCl, 5 mM  $\text{CaCl}_2$ , 10%  $\text{D}_2\text{O}$ , 0.02 %  $\text{NaN}_3$



### X-Filtered NOESY NMR Standard

Catalog No.	Label
NEX-XF1-U-0	unlabeled
NEX-XF1-N-0	( $^{15}\text{N}$ , 95%)
NEX-XF1-CN-5-0	( $^{13}\text{C}$ , 5%; $^{15}\text{N}$ , 95%)
NEX-XF1-CN-0	( $^{13}\text{C}$ , 95%; $^{15}\text{N}$ , 95%)
NEX-XF1-CDN-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%)
NEX-XF1-ILV-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILV)
NEX-XF1-ILVY-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILVY)

### X-Filtered NOESY NMR Standard, His-Tagged

NEX-XF1-HIS-U-0	unlabeled
NEX-XF1-HIS-N-0	( $^{15}\text{N}$ , 95%)
NEX-XF1-HIS-CN-5-0	( $^{13}\text{C}$ , 5%; $^{15}\text{N}$ , 95%)
NEX-XF1-HIS-CN-0	( $^{13}\text{C}$ , 95%; $^{15}\text{N}$ , 95%)
NEX-XF1-HIS-CDN-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%)
NEX-XF1-HIS-ILV-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILV)
NEX-XF1-HIS-ILVY-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILVY)

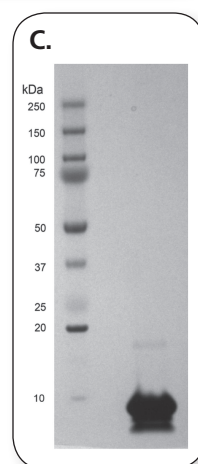
**A.** 2D  $^1\text{H}$ - $^1\text{H}$  plane of  $^1\text{H}$ ,  $^{13}\text{C}$  edited  $^1\text{H}$ ,  $^{12}\text{C}$  X-filtered NOESY

**B.**  $^1\text{H}$ - $^{15}\text{N}$  HSQC of NEX-XF1

**C.** SDS-PAGE GEL NEX-XF1

### Protein Sequence

PKIIEAVYENGVFKPLQKVDLKEGERVKIKLELKVPEIDLGEPVS  
VEEIKKIRDGTWMSLEHHHHHH



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# Ubiquitin (NEX-UB1)

NEX-UB1 is a small 8.8 kDa monomeric protein for which multiple sets of resonance assignments (BMRB database) and 3D structures (PDB database) are publicly available. This protein standard is uniformly  $^{15}\text{N}$ ,  $^{13}\text{C}$ -enriched. Ubiquitin has been used as an industry-wide standard in the protein NMR field for many years.

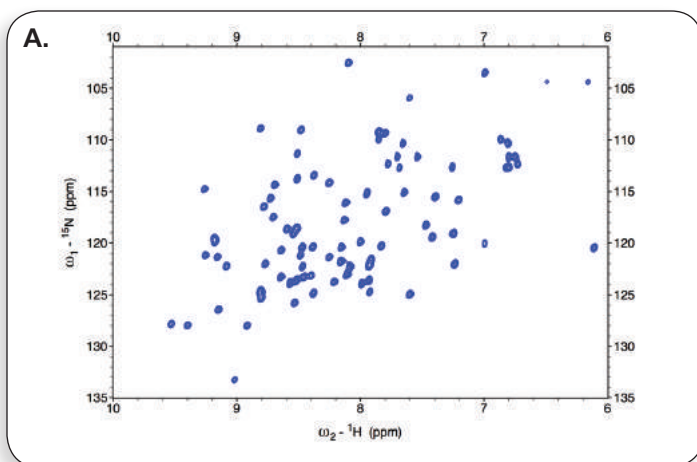
## NEX-UB1 sample formulation:

**NEX-UB1:** Uniformly  $^{15}\text{N}$ ,  $^{13}\text{C}$ -labeled ubiquitin in 90%  $\text{H}_2\text{O}$ ; 10%  $\text{D}_2\text{O}$   
10 mM sodium phosphate buffer, pH 6.5

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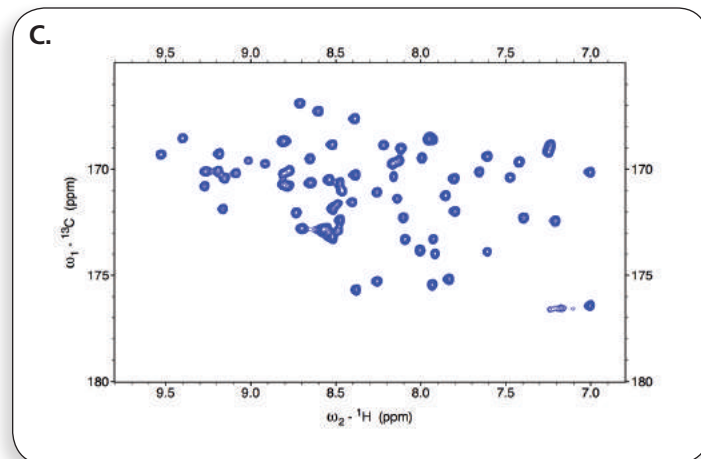
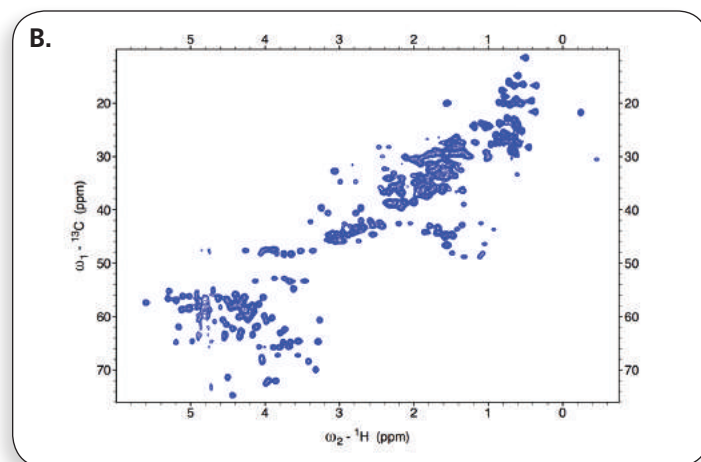


A.  $^1\text{H}$ ,  $^{15}\text{N}$  HSQC of NEX-UB1

B.  $^{13}\text{C}$ - $^1\text{H}$  HSQC of NEX-UB1

C. CO-NH 2D plane of HNCO triple-resonance experiment of NEX-UB1

D. SDS-PAGE GEL NEX-UB1



### Ubiquitin (Human)

Catalog No.	Label
NEX-UB1-U-0	unlabeled
NEX-UB1-N-0	( $^{15}\text{N}$ , 95%)
NEX-UB1-CN-5-0	( $^{13}\text{C}$ , 5%; $^{15}\text{N}$ , 95%)
NEX-UB1-CN-0	( $^{13}\text{C}$ , 95%; $^{15}\text{N}$ , 95%)
NEX-UB1-CDN-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%)
NEX-UB1-ILV-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILV)
NEX-UB1-ILV-FY-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILV-FY)

### His-Ubiquitin (Human)

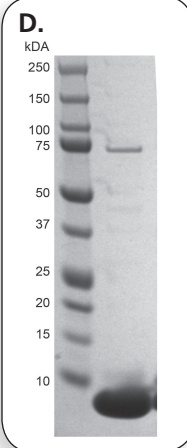
NEX-UB1-HIS-U-0	unlabeled
NEX-UB1-HIS-N-0	( $^{15}\text{N}$ , 95%)
NEX-UB1-HIS-5-0	( $^{13}\text{C}$ , 5%; $^{15}\text{N}$ , 95%)
NEX-UB1-HIS-CN-0	( $^{13}\text{C}$ , 95%; $^{15}\text{N}$ , 95%)
NEX-UB1-HIS-CDN-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%)
NEX-UB1-HIS-ILV-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILV)
NEX-UB1-HIS-ILV-FY-0	( $^{13}\text{C}$ , 95%; D, 95%; $^{15}\text{N}$ , 95%; $^{13}\text{CH}_3$ -ILV-FY)

### Protein Sequence after TEV Cleavage

SHMQIFVKLTGKTTITLEVEPSDTIENVKAKIQDKEGIPPDQQR  
LIFAGKQLEDGRTLSDYNIQKESTLHLVLRLLGG

### Protein Sequence before TEV Cleavage

MGHHHHHHENLYFQSHMQIFVKLTGKTTITLEVEPSDTIEN  
VKAKIQDKEGIPPDQQRLLIFAGKQLEDGRTLSDYNIQKESTL  
HLVLRLLGG



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3/15 Supersedes all previously published literature