Biomolecular NMR

Deuterated Detergents and Phospholipids for Membrane Proteins

Membrane proteins can be divided into three categories:

1. Integral membrane proteins, which can penetrate the lipid bilayer
2. Peripheral membrane proteins, which are external and bound through noncovalent interactions
3. Lipid-anchored proteins, which are external but bound with covalent bonds.

There is a great interest in determining structure of integral membrane proteins due to the importance of these proteins in participating in cellular processes. Despite the significant, functional importance of membrane proteins, the structural biology has been particularly challenging, which is reflected by the low number of determined membrane protein structures.[1]

The determination of the structure and dynamics of membrane proteins using NMR requires samples containing protein that is properly folded. Fortunately, membrane proteins often keep native-like structures in detergent micelles. Deuterated solubilization agents, such as detergents, often make NMR investigations easier compared to using unlabeled agents. In some cases, such as methyl labeling, deuterated reagents of this type are required. CIL is pleased to offer the following deuterated detergents and phospholipid agents for use with membrane proteins.

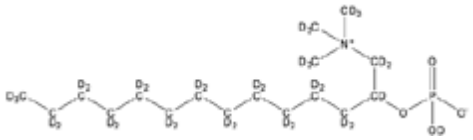
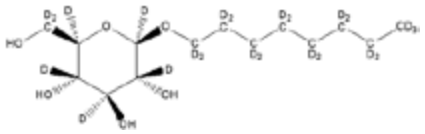
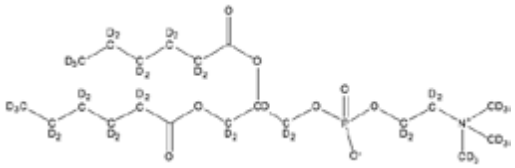
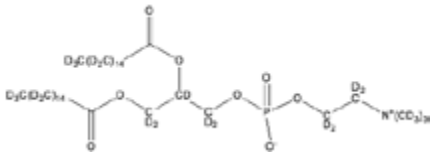
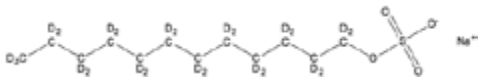
Reference

1. There are 493 unique membrane protein structures as of August 3, 2014. See http://blanco.biomol.uci.edu/index.shtml for more information.

"CIL has been a strong supporter of NMR methods of development over the years, providing critical isotope-enriched reagents for research and development, without which many of the recent advances in biomolecular NMR would simply not have been possible. In particular, the broad biological impact and tremendous success of the multidimensional triple-resonance biomolecular NMR would not have been achieved without the high-quality and broadly accessible reagents that CIL has provided to the scientific community over the last 20 years."

Gaetano Montelione, PhD
Professor of Molecular Biology and Biochemisty
Rutgers University Director of the
Northeast Structural Genomics Consortium

Deuterated Detergents and Phospholipids

Catalog No.	Description
DLM-2274	Dodecylphosphocholine (D_{38}, 98%) 
DLM-6726	*N*-Octyl β-Glucoside (D_{24}, 98%) 
DLM-4341	DL-A-Phosphatidylcholine, dihexanoyl (D_{40}, 98%) (DHPC) CP 95% 
DLM-8256	DL-A-Phosphatidylcholine, dipalmitoyl (DPPC) (D_{80}, 98%) CP 95%+ 
DLM-197	Sodium dodecyl sulfate (D_{25}, 98%) 

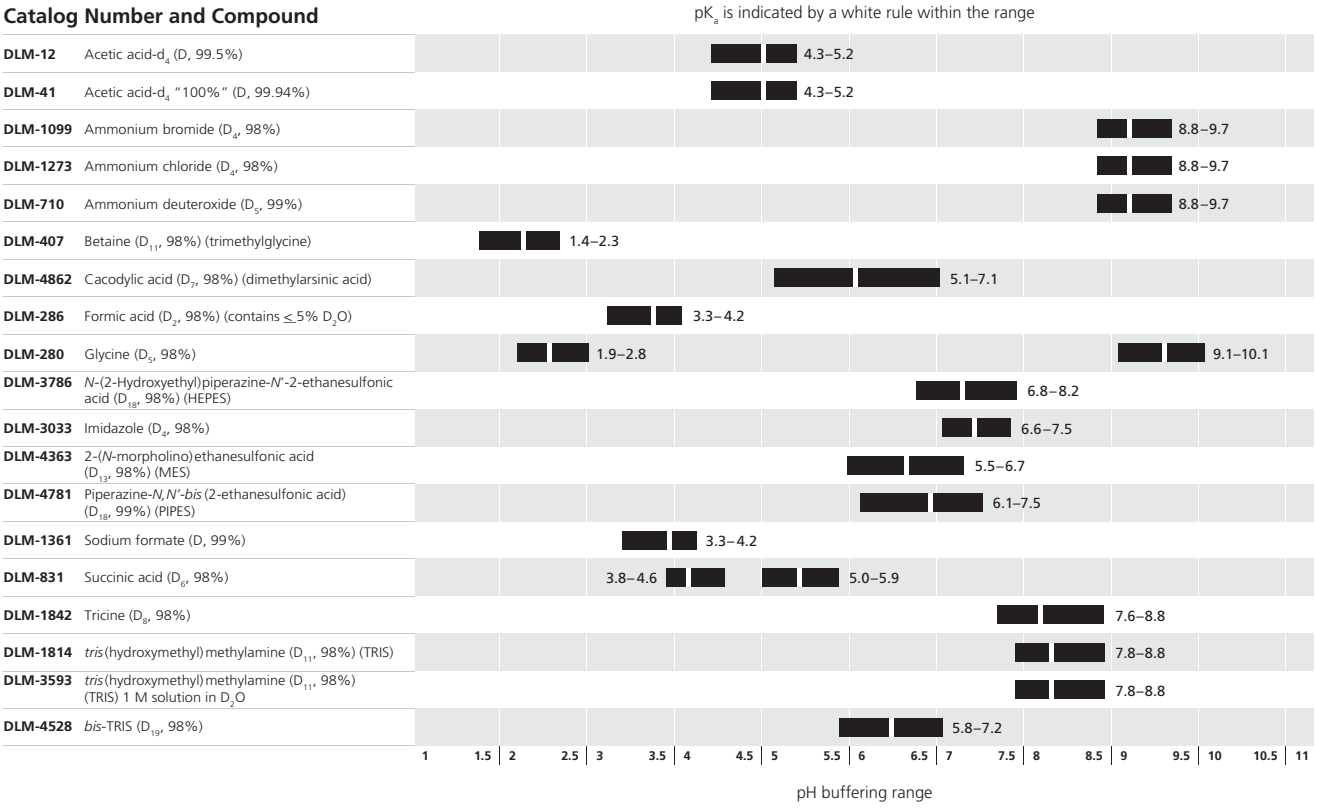
Deuterated Buffers

CIL offers a wide selection of deuterated buffers for use with aqueous solutions.

Catalog No.	Description
DLM-12	Acetic acid-d_4 (D, 99.5%)
DLM-41	Acetic acid-d_4 "100%" (D, 99.93%)
DLM-1099	Ammonium bromide (D_4, 98%)
DLM-1273	Ammonium chloride (D_4, 98%)
DLM-710	Ammonium deuteroxide (D_5, 99%) (~25% in soln D_2O)
DLM-407	Betaine (D_{11}, 98%)
DLM-4862	Cacodylic acid (D_7, 98%)
DLM-286	Formic acid (D_2, 98%) (<5% D_2O)
DLM-280	Glycine (D_5, 98%)
DLM-3786	HEPES (D_{18}, 98%)

Catalog No.	Description
DLM-3033	Imidazole (D_4, 98%)
DLM-4363	MES (D_{13}, 98%)
DLM-4781	PIPES (D_{18}, 98%)
DLM-1361	Sodium formate (D, 98%)
DLM-831	Succinic acid (D_6, 98%)
DLM-1842	Tricine (D_8, 98%)
DLM-4779	Trimethylamine *N*-oxide (D_9, 98%)
DLM-1814	TRIS (D_{11}, 98%)
DLM-3593	TRIS (D_{11}, 98%) 1 M in D_2O
DLM-4528	*bis*-TRIS (D_{19}, 98%)

pH Buffering Range Chart



Isotope-Labeled Protein Standards

CIL is pleased to offer isotope-enriched proteins for use as standards in NMR spectroscopy. CIL is also happy to offer new and exciting protein standards manufactured by Nexomics Biosciences, Inc., a New Jersey-based contract research organization that specializes in a broad array of gene-to-structure services for the biopharmaceutical community.

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

- ^{1}H-^{15}N HSQC (^{15}N-labeled proteins)
- ^{1}H-^{13}C HSQC (^{13}C-labeled proteins)
- CO-NH projection of 3D HNCO (^{15}N,^{13}C-labeled proteins)
- SDS PAGE (for all labeled proteins)
- MALDI-TOF (for all labeled proteins)

Isotope-enriched protein standards are ideal for:

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

All protein standards offered by CIL have been chosen for the high-quality spectra they produce and the excellent long-term stability they exhibit.

Protein and Peptide Standards

The Chicken α-Spectrin SH3 Domain is available in microcrystalline form (in an ammonium sulfate emulsion) or as a 9 mg/mL solution (10% D_2O/90% H_2O containing 0.02% NaN_3, pH 3.5). A full technical data package containing 2D-NMR data and peak assignments accompanies every order for the Chicken α-Spectrin SH3 Domain. Every order for the GB1 is accompanied by a ^{1}H-^{15}N-HSQC spectrum.

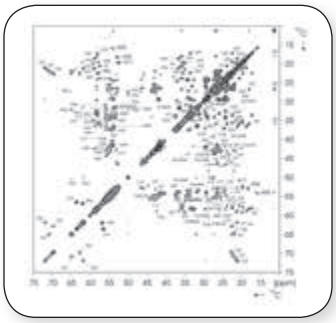
GB1 is offered as a *N*-terminal, tobacco etch virus (TEV)-cleavable 6xHis-tag as a 1.5 mM solution in 137 mM NaCl, 2.7 mM KCl, 8.1 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 0.02% NaN_3, 0.1 mM TSP in 10% D_2O/90% H_2O, pH 6.5. GB1 is noted for its excellent stability at elevated temperatures.

Catalog No.	Description
CLM-8227	SH3 Domain Protein (U-^{13}C, 98%)
NLM-6839	SH3 Domain Protein (U-^{15}N, 98%)
NLM-6839-S	SH3 Domain Protein (U-^{15}N, 98%) (9 mg/mL solution)
CNLM-6840	SH3 Domain Protein (U-^{13}C, 98%; U-^{15}N, 98%) (microcrystalline slurry)
CNLM-6840-S	SH3 Domain Protein (w/ 0.01% sodium azide) (U-^{13}C, 98%; U-^{15}N, 98%) (9 mg/mL solution)
CDNLM-6841	SH3 Domain Protein (U-^{13}C, 98%; U-D, 98%; U-^{15}N, 98%)
CDNLM-6841-S	SH3 Domain Protein (9 mg/mL solution) (U-^{13}C, 98%; U-D, 98%; U-^{15}N, 98%)
CNLM-2408	GFL Peptide Standard (^{13}C, 98%; ^{15}N, 96-99%) 1 mM in DMSO-d_6

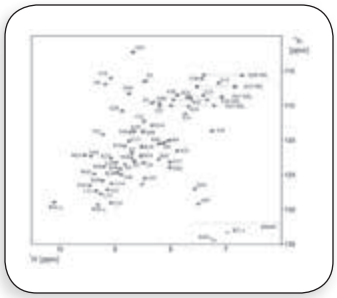
CIL His-Tagged Protein Standards

Catalog No.	Description
CNLM-8663	His-GB1 (^{13}C, 98%+; ^{15}N, 98%+) 1.5 mM in PBS, pH 6.5, 0.02% sodium azide

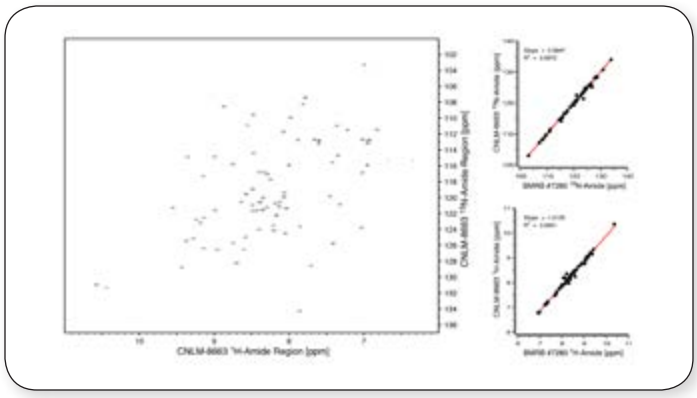
Product	Amino Acid Length
Chicken α-Spectrin SH3 Domain	62 residues
MDETGKELVL ALYDYQEKSP REVTMKKGDI LTLLNSTNKD WWKVEVNDRQ GFVPAAYVKK LD	
His6x-GB1 (β-1 immunoglobulin domain of protein G)	71 residues
MHHHHHHGEN LYFQSMQYKL ILNGKTLKGE TTTEAVDAAT AEKVFKQYAN DNGVDGEWTY DDATKTFTVT E	
GFL Peptide	8 residues
YG**GFL**RRI (bold indicates labeled residues)	



(Right) ^{13}C-^{13}C solid-state NMR spectrum SH3 protein (U-^{13}C, U-^{15}N).



(Left) ^{1}H-^{15}N-HSQC NMR spectrum of SH3 Domain Protein (U-^{13}C, U-^{15}N).



^{1}H,^{15}N-HSQC of 1.5 mM Immunoglobulin-Binding Domain B1 of Streptococcal Protein G (U-^{13}C, 99%; U-^{15}N, 99%) containing an *N*-terminal His6-tag and TEV protease cleavage site (CNLM-8663-CA, Lot# 20110209). The ^{15}N-amide (top, right) and ^{1}H-amide (bottom, right) assignments of CNLM-8663-CA show excellent correlation with those previously reported in the Biological Magnetic Resonance Bank for GB1 (BMRB #7280) lacking the His6-TEV leader sequence.

Maltose Binding Protein (NEX-MBP)

NEX-MBP is a 44.9 kDa monomeric protein with multiple sets of resonance assignments (BMRB database) and 3D structures (PDB database) that are publicly available. This product is uniformly D,^{15}N,^{13}C-enriched with selective incorporation of protons into methyl groups of Ile-δ1, Leu-δ and Val-γ side chains. As nonuniform sampling (NUS) and other NMR techniques emerge to push the size limitations of NMR to new boundaries, larger 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 D,^{15}N,^{13}C and ILV methyl ^{1}H,^{13}C MBP in 10% D_2O, 0.02% NaN_3, 20 mM sodium phosphate @ pH 7.2

NEX-MBP2: Closed Conformation

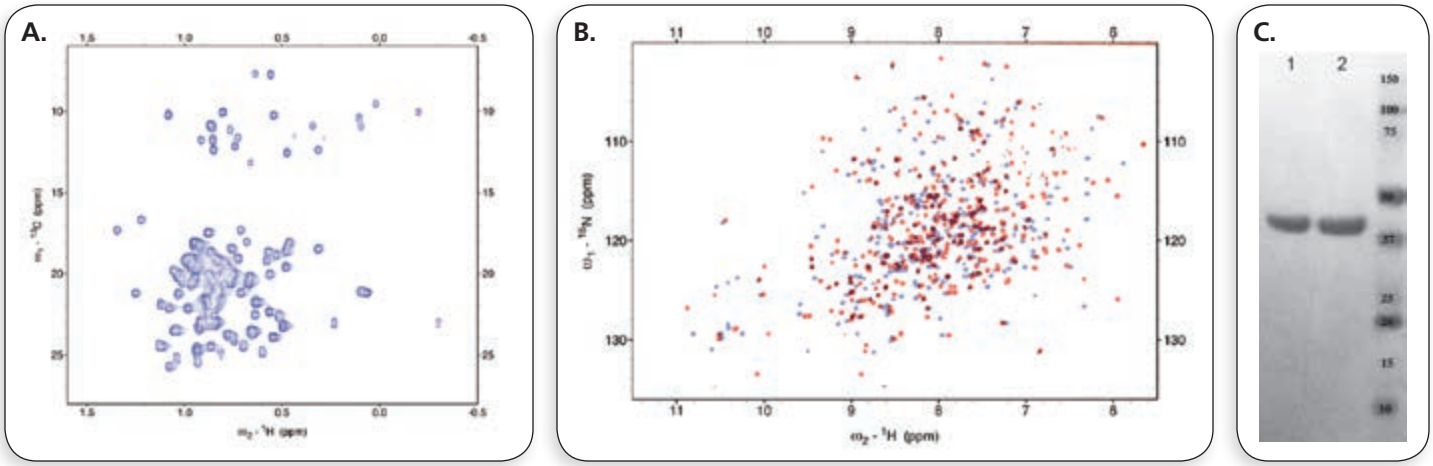
0.5 mM D,^{15}N,^{13}C and ILV methyl ^{1}H,^{13}C MBP with 3 mM maltotriose, 10% D_2O, 0.02% NaN_3, 20 mM sodium phosphate @ pH 7.2

NEX-MBP3: Open Conformation

0.5 mM D,^{15}N,^{13}C and ILV methyl ^{1}H,^{13}C MBP with 2 mM β-cyclodextrin, 10% D_2O, 0.02% NaN_3, 20 mM sodium phosphate @ pH 7.2

Available in a sealed NMR tube upon request

Contact CIL for different packaging configurations



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

Catalog No.	Label
NEX-MBP1-U-0	unlabeled
NEX-MBP1-N-0	(^{15}N, 95%)
NEX-MBP1-CN-5-0	(^{13}C, 5%; ^{15}N, 95%)
NEX-MBP1-CN-0	(^{13}C, 95%; ^{15}N, 95%)
NEX-MBP1-CDN-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%)
NEX-MBP1-ILV-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%; $^{13}CH_3$-ILV)
NEX-MBP1-ILVFY-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%; $^{13}CH_3$-ILVFY)

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

NEX-MBP2-U-0	unlabeled
NEX-MBP2-N-0	(^{15}N, 95%)
NEX-MBP2-CN-5-0	(^{13}C, 5%; ^{15}N, 95%)
NEX-MBP2-CN-0	(^{13}C, 95%; ^{15}N, 95%)
NEX-MBP2-CDN-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%)
NEX-MBP2-ILV-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%; $^{13}CH_3$-ILV)
NEX-MBP2-ILVFY-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%; $^{13}CH_3$-ILVFY)

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

NEX-MBP3-U-0	unlabeled
NEX-MBP3-N-0	(^{15}N, 95%)
NEX-MBP3-CN-5-0	(^{13}C, 5%; ^{15}N, 95%)
NEX-MBP3-CN-0	(^{13}C, 95%; ^{15}N, 95%)
NEX-MBP3-CDN-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%)
NEX-MBP3-ILV-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%; $^{13}CH_3$-ILV)
NEX-MBP3-ILVFY-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%; $^{13}CH_3$-ILVFY)

A. **^{13}C,^{1}H HSQC NEX-MBP3 "open" conformation**
B. **"Open" (blue) and "closed" (red) superposition**
C. **SDS-PAGE GEL NEX-MBP**
NEX-MBP3 β-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

MKIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIIFWAH
DRFGGYAQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYNKDLLPNPPKTWEE
IPALDKELKAKGKSALMFNLQEPYFTWPLIAADGGYAFKYENGKYDIKDVGVDNAGAKAGLTFL
VDLIKNKHMNADTDYSIAEAAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKP
FVGVLSAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEELAKDPRIAATMEN
AQKGEIMPNIPQMSAFWYAVRTAVINAASGRQTVDEALKDAQTRITK

(continued)

X-Filtered NOESY NMR Standard (NEX-XF1)

In an X-filtered experiment, only NOEs between $^{15}N/^{13}C$-^{1}H and $^{14}N/^{12}C$-^{1}H (e.g. interchain NOEs) protons are observed. NOEs between protons connected to $^{15}N,^{13}C$ are filtered (intrachain NOEs). When a uniformly double-labeled protein sample is mixed with a natural-abundance protein sample, the interface will give rise to the only observable NOESY crosspeaks. This powerful strategy enables the spectroscopist to discern intra from inter NOESY crosspeaks, 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 14 kDa protein (*A. fulgidus* antoxin 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 $^{15}N,^{13}C$-enriched protein (25% homodimer unlabeled; 50% heterodimer unlabeled/labeled; 25% homodimer labeled) and is perfect to set up X-filtered NOESY experiments.

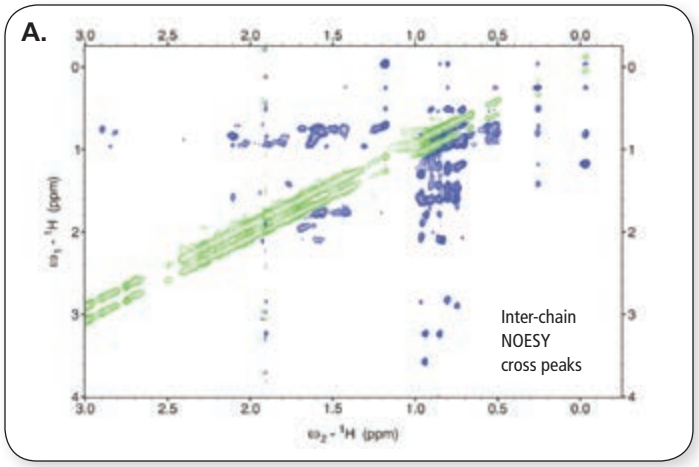
Available in a sealed NMR tube upon request

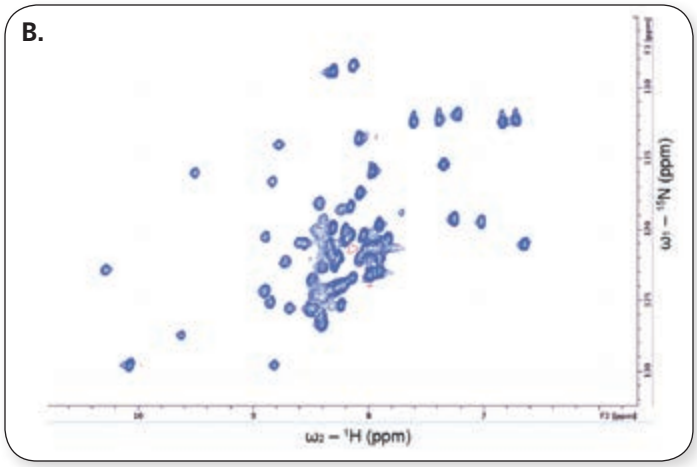
Contact CIL for different packaging configurations

NEX-XF1 homodimer sample formulation:

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

1 mM protein, 20 mM NH_4OAc pH 5.5, 100 mM NaCl, 5 mM $CaCl_2$, 10 mM DTT, 10% D_2O, 0.02 % NaN_3

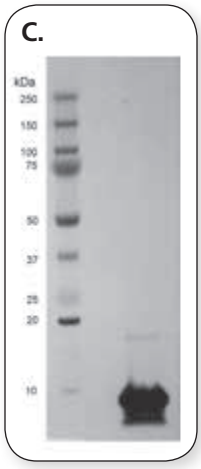




A. **2D ^{1}H-^{1}H plane of $^{1}H,^{13}C$ edited $^{1}H,^{12}C$ X-filtered NOESY**

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

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



X-Filtered NOESY NMR Standard

Catalog No.	Label
NEX-XF1-U-0	unlabeled
NEX-XF1-N-0	(^{15}N, 95%)
NEX-XF1-CN-5-0	(^{13}C, 5%; ^{15}N, 95%)
NEX-XF1-CN-0	(^{13}C, 95%; ^{15}N, 95%)
NEX-XF1-CDN-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%)
NEX-XF1-ILV-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%; $^{13}CH_3$-ILV)
NEX-XF1-ILVFY-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%; $^{13}CH_3$-ILVFY)

X-Filtered NOESY NMR Standard, His-Tagged

NEX-XF1-HIS-U-0	unlabeled
NEX-XF1-HIS-N-0	(^{15}N, 95%)
NEX-XF1-HIS-CN-5-0	(^{13}C, 5%; ^{15}N, 95%)
NEX-XF1-HIS-CN-0	(^{13}C, 95%; ^{15}N, 95%)
NEX-XF1-HIS-CDN-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%)
NEX-XF1-HIS-ILV-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%; $^{13}CH_3$-ILV)
NEX-XF1-HIS-ILVFY-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%; $^{13}CH_3$-ILVFY)

Protein Sequence

PKIIEAVYENGVFKPLQKVDLKEGERVKIKLELKVEPIDLGEPVS
VEEIKKIRDGTWMSSLEHHHHHH

Ubiquitin (NEX-UB1)

Powder form available

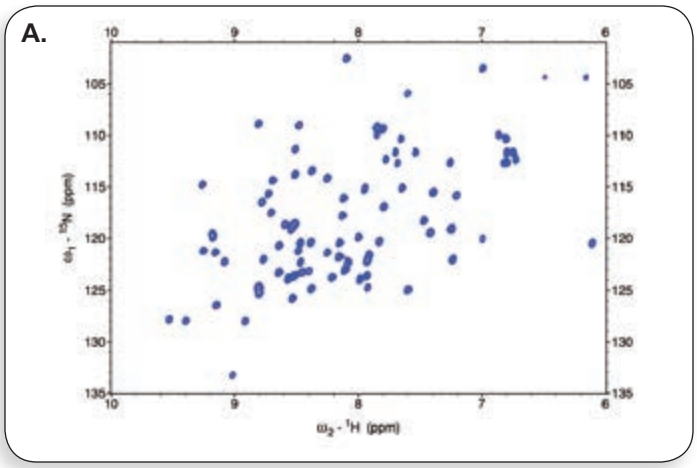
Available in a sealed NMR tube upon request

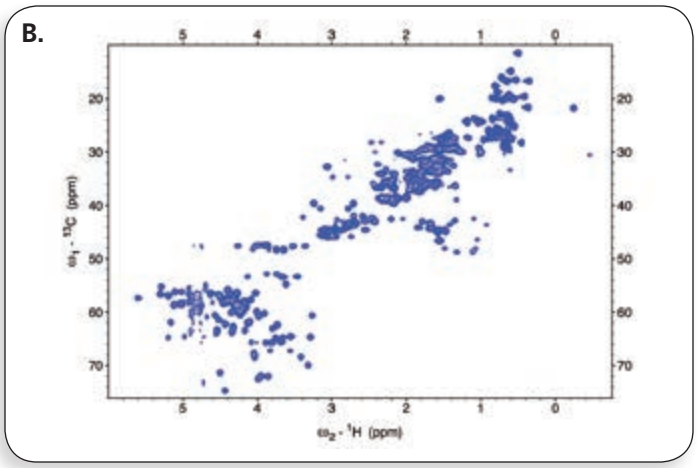
Contact CIL for different packaging configurations

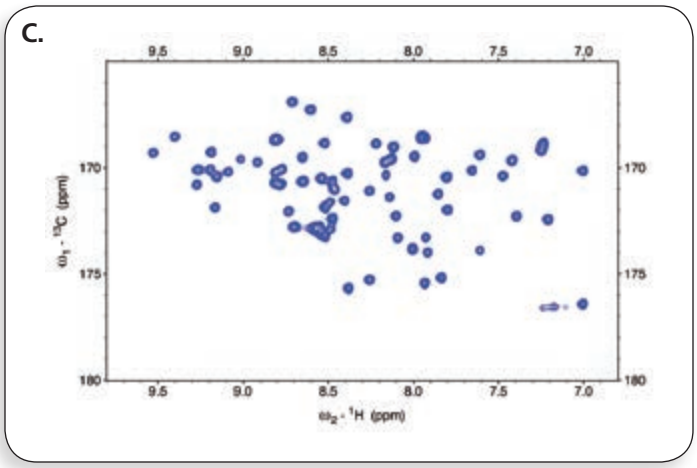
NEX-UB1 is a small 8.8 kDa monomeric protein for which multiple sets of resonance assignments (BMRB database) and 3D structures (PDB database) are publicly available. This protein standard is uniformly ^{15}N,^{13}C enriched. Ubiquitin has been used as an industry-wide standard in the protein NMR field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

NEX-UB1 sample formulation:

NEX-UB1: Uniformly ^{15}N,^{13}C-labeled ubiquitin in 90% H_2O; 10% D_2O
10 mM sodium phosphate buffer, pH 6.5







A. **^{1}H,^{15}N HSQC of NEX-UB1**
B. **^{13}C-^{1}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}N, 95%)
NEX-UB1-CN-5-0	(^{13}C, 5%; ^{15}N, 95%)
NEX-UB1-CN-0	(^{13}C, 95%; ^{15}N, 95%)
NEX-UB1-CDN-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%)
NEX-UB1-ILV-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%; $^{13}CH_3$-ILV)
NEX-UB1-ILVFY-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%; $^{13}CH_3$-ILVFY)

His-Ubiquitin (Human)

NEX-UBI-HIS-U-0	unlabeled
NEX-UBI-HIS-N-0	(^{15}N, 95%)
NEX-UBI-HIS-5-0	(^{13}C, 5%; ^{15}N, 95%)
NEX-UBI-HIS-CN-0	(^{13}C, 95%; ^{15}N, 95%)
NEX-UBI-HIS-CDN-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%)
NEX-UBI-HIS-ILV-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%; $^{13}CH_3$-ILV)
NEX-UBI-HIS-ILVFY-0	(^{13}C, 95%; D, 95%; ^{15}N, 95%; $^{13}CH_3$-ILVFY)

Protein Sequence after TEV Cleavage

SHMQIFVKTLTGKTITLEVEPSDTIENVKAKIQDKEGIPPDQQR LIFAGKQLEDGRTLSDYNIQKESTLHLVLRLRGG

Protein Sequence before TEV Cleavage

*MGHHHHHHENLYFQ*SHMQIFVKTLTGKTITLEVEPSDTIEN VKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLSDYNIQKESTL HLVLRLRGG

D.