UPON RECEIPT OF NMR KIT

1. Inspect each sample: Upon receipt of the NMR kit, please inspect each sample to assure that each is near +10 °C ±10 °C. The NMR kit is designed to maintain 'cool' temperatures for 3 - 4 days. While the sample will tolerate brief periods at room temperature, for purposes of comparability the sample should not be kept at this temperature. If the temperature of the package is found to be 'hot' (> 30 °C) upon arrival, please request a new sample.

2. Contents of the NMR Kit

- **A. 100% methanol-d4 sealed sample**
- **B. U-^{15}N, 20%-^{13}C NIST-Fab system suitability sample**
- **C. Unlabeled NIST-Fab sample**

3. Storage: After sample inspection, please store samples in vertical position in a refrigerator at +5 °C ±5 °C when the samples are not being used to acquire experiments in your spectrometer.

If a problem arises with any sample, please request a new one. See Page 2 for more details.

The NIST NMR Interlaboratory Study

Welcome: Thank you for participating in the NIST NMR interlaboratory study. Your participation is a critical element to the success of this study. We at NIST are grateful for your interest in the harmonization of measurement protocols for the assessment of higher order structure. This document is organized into the following sections:

Please contact Robert Brinson (robert.brinson@nist.gov) and John Marino (john.marino@nist.gov) if questions arise.

Proposed Timeline for the NMR Study:

- **2016 June 3, 6, 7** – distribution of the NMR kits
- **2016 September 15** – reporting of NMR results to National Association of Proficiency Testing (NAPT)
- **2016 November** – release of initial data analysis by NIST
- **2017 February** – submission of manuscript for author and institutional reviews
- **2017 May** – submission of manuscript to journal

If a problem arises with any sample in the NMR kit: Each NMR kit contains multiple samples. If any of these samples are in any way defective, new sample(s) may be requested. Figure 1 outlines the simple procedure for requesting a new NMR sample or kit. Adherence to this procedure is necessary so that the possibility of bias and unwanted traceability are removed. In the case that your sample arrives warm, please request an entirely new kit. If you suspect that a laboratory error has corrupted any of your samples, please request a new sample. No justification or explanation is required when requesting a new NMR sample or kit—just ask.

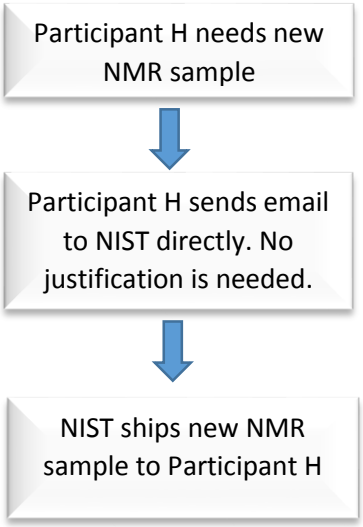

Figure 1. Procedure for requesting a new NMR sample.

I. Scope of the Study: Harmonization of NMR Methods

Critical quality attributes (CQA) are significant measurement parameters of a medical product that impact both product safety and efficacy and are essential characteristics that are linked to positive public health outcomes. One CQA, higher order structure, is directly coupled to the function of protein biologics (biopharmaceuticals), and deviations in this CQA may be linked to pathological functions (e.g., immunogenicity or

toxicity). NMR spectra can yield structural fingerprints for a protein biologic at atomic resolution that are intrinsically dependent on higher order structure. While NMR spectral methods are well established for small molecules, peptides and small proteins, these approaches are far from standard or routine for proteins above 30 kDa in size, such as monoclonal antibodies (mAbs).

The primary goal of this project is to use the Fab domain from the NISTmAb (see Section II) to demonstrate the robustness of the NMR measurement and to validate NMR structural fingerprinting measurements for the assessment of higher order structure of large protein biologics and/or domains from these proteins. The validation of NMR methods for the characterization of the higher order structure of mAbs is specifically targeted due to the large interest of the pharmaceutical industry in using mAbs as platforms for therapeutic development.

A secondary goal of this study involves the validation of an acquisition technique known as non-uniform sampling (NUS). The NMR measurement has the reputation of high sample consumption and time intensive experiments. For the former, the mAb drug substances are often formulated at high enough concentration that sample consumption is less of a concern for this application. For the latter concern, any NMR method using traditional uniform sampling acquisition would still be time intensive. However, NUS has the potential to greatly reduce the overall measurement time for all biopharmaceuticals by 50% for a 2D NMR spectrum.[1] Indeed, by pairing the NUS technique with a SOFAST or BEST pulse sequence, the NIST lab reduced the overall measurement time on the Fc and Fab domains to approximately 30 minutes for a ^{13}C HSQC on the methyl region.[2] Due to the large size of this study, a standard gradient selected HSQC will be used, although optional SOFAST measurements may also be collected. See Section III for more details.

II. Properties of the Fab Domain Derived from the NISTmAb

The test protein for the NMR interlaboratory study is the Fab domain from the NISTmAb (Figure 2)—hereafter referred to as NIST-Fab—that was enzymatically produced from the NIST candidate reference material #8670 IgG1κ. The NISTmAb was donated to NIST in a formulated state of 100 mg/mL in 25mM L-histidine, pH 6.0, is considered a drug-like substance. It will shortly be released as a reference material. The NIST-Fab contains no glycosylation and has homogeneous composition; hence, in the absence of the Fc domain and intact mAb, it is a homogeneous material and may be considered to be a Type A material.[3]

The unlabeled NIST-Fab protein in your kit was produced in quantity by application to immobilized papain cross-linked on agarose beaded support (Figure 2). Following the cleavage of the NISTmAb into the Fab and Fc domains, NIST-Fab was purified with a protein A affinity column. It was subsequently passed through a 100 kDa MWCO Amicon filter to remove high molecular weight impurities followed by a 30 kDa MWCO Amicon filter to remove low molecular weight contaminants. During the final filtration step, NIST-Fab was buffer exchanged into

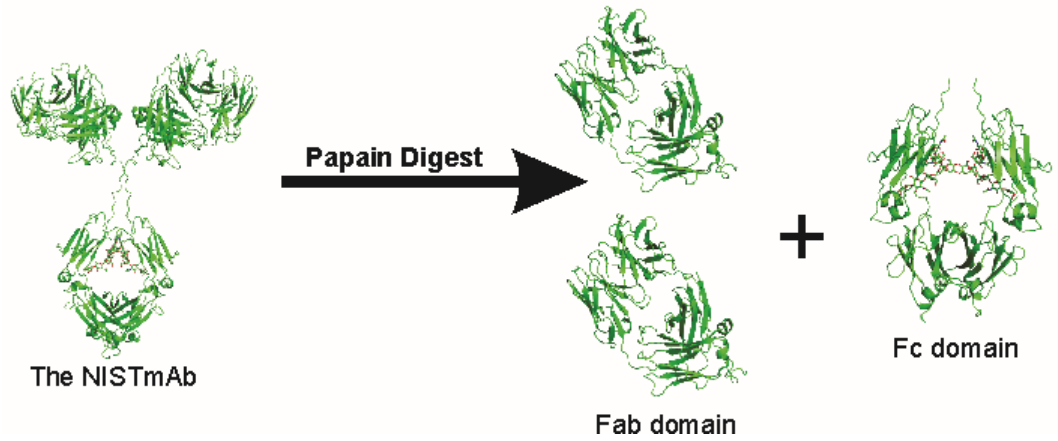

Figure 2. Schematic representation of the papain cleavage of the NISTmAb into its constituent Fab and Fc domains.

25 mM bis-tris-d19, pH 6.0, in 95% H_2O and 5% D_2O. The NIST-Fab was pooled into one fraction at a final concentration of 429 µM in 25 mM bis-tris-d19, pH 6.0.

The system suitability sample, uniformly labeled (U)-^{15}N, 20%-^{13}C-NIST-Fab, was produced by expression in *P. pastoris.* The heavy and light chains, corresponding to the sequence of the Fab domain obtained after papain cleavage of the NISTmAb, were produced using a bicistronic expression vector that secretes the folded protein product into the growth media. Isotope labeling was carried out using ^{15}N-ammonium sulfate as the sole nitrogen source and ^{13}C-methanol as the labeled carbon source. The product was purified using an IgG-CH1 affinity column. The purified U-^{15}N, 20%-^{13}C-NIST-Fab was buffer exchanged into 25 mM bis-tris-d19, pH 6.0, and adjusted with the same buffer to the final concentration of 53 µM. All final quality control checks were performed on the final, pooled sample.

This sample was sent to NIST-IBBR in Rockville, MD, USA for distribution to all partners. All samples will be packaged with cold packs and shipped from NIST-IBBR via FedEx, unless directed otherwise.

III. NMR Interlaboratory Data Collection Protocol

A. Contents of the Sample Kit: The NMR Sample Kit is designed to minimize the material costs to participants. All NMR microtubes for the appropriate vendor (*i.e.*, Agilent, Bruker Biospin, or JEOL Resonance, Inc) were purchased from Shigemi, Inc (Allison Park, PA, USA). The NMR tubes come pre-loaded with sample and with an air bubble to allow for water expansion in the event the samples freeze during shipment. As per normal protocol, please remove the air bubble between the plunger and sample before performing any measurements.

1. 100% Methanol-d4. This sample is for temperature calibration.

2. 53 µM U-^{15}N, 20%-^{13}C NIST-Fab system suitability sample. This NIST-Fab sample was expressed in *P. pastoris* at Health Canada in Ottawa, Ontario, Canada. This sample will serve as the benchmark for all measurements.

3. 429 µM Unlabeled NIST-Fab. This NIST-Fab sample was derived from the NISTmAb candidate reference material #8670. The unlabeled fragment was generated at NIST-IBBR in Rockville, MD, USA.

Prior to the first measurements, both NIST-Fab samples need to be pre-warmed to 37 °C for a minimum of 30 minutes for the purpose of degassing. After this period, please check and adjust the shigemi tube to remove any air bubble(s) that may have arisen. This procedure should mitigate the possibility of an air bubble forming during your experiments.

B. Temperature Calibration, 100% Methanol-d4. The standard samples of 100% methanol and 100% ethylene glycol are not suitable for the high sensitivity of cryogenically cooled probes.[4] To overcome this limitation, we have provided a sealed sample of perdeuterated methanol for this purpose.

1. Run three 1D ^{1}H experiments on the 5 mm 100% methanol-d4 sample at 310 K. Please calculate the normalized temperature using the following equation:

$$T = -16.7467 \times (\Delta\delta)^2 - 52.5130 \times \Delta\delta + 419.1381$$

where Δδ is the chemical shift difference (in ppm) between the $-CH_3$ and OH resonances, and T is the absolute temperature of the sample in Kelvin. For instruments manufactured by Bruker Biospin, the command <calctemp> utilizes this equation for the calculation of the absolute temperature.

2. Adjust the temperature of the probe so that it is **310 K ± 0.1 K, corresponding to a chemical shift difference of 1.428 ppm**. Use this calibration for all your measurements.

Temperature calibration is vital for comparability assessment in the NMR interlaboratory study.

C. Summary of Required Experiments

Please perform experiments with a sufficient number of scans per increment to achieve an average minimum S/N of 10:1. In Table 1, we list the NIST instruments and their ^{1}H and ^{13}C sensitivities as determined from 0.1% ethyl benzene and ASTM samples, respectively. These data will allow each participant to gauge how long an experiment will likely take on their NMR system. Total measurement time of gsHSQC experiments from NIST 600 MHz are given in Table 2. A separate guidance has been prepared that provides instructions of how to determine overall experimental time from the first FID (Appendix I).

All experiments will be performed with the $^{1}H,^{15}N$ or $^{1}H,^{13}C$ gradient selected heteronuclear single quantum coherence experiments (gsHSQC, Figures 3 and 4). The ^{1}J coupling constant for the amide and methyl groups should be set to 93 Hz and 145 Hz, respectively. See Tables 2 and 3 for acquisition parameters. Frequency referencing discrepancies will be rectified during processing with NMRPipe.

D. Experimental List– All Experimental Parameters are given in Tables 3 – 6

1. $^{1}H,^{15}N$-gsHSQC: U-^{15}N, 20%-^{13}C-NIST-Fab (Tables 3 – 4)

a. Uniform sampling (US), acquisition time in F1 = 20 ms

2. $^{1}H,^{13}C$-gsHSQC: U-^{15}N, 20%-^{13}C-NIST-Fab (Tables 5 – 6)

a. US 128 total points in F1 (64 complex points)
b. 50% NUS Schedule 1, 128 total reconstructed pts in F1
c. US acquisition time in F1 = 25 ms (0.5 * T2)
d. 50% NUS Schedule 2 Field Dependent, acquisition time in F1 = 25 ms (0.5 * T2)
e. 50% Time Equivalent (TE) NUS, Schedule 2

3. $^{1}H,^{13}C$-gsHSQC: unlabeled NIST Fab Fragment, derived from the NISTmAb (Tables 5 – 6)

a. US acquisition time in F1 = 25 ms (0.5 * T2)
b. 50% NUS Schedule 2, acquisition time in F1 = 25 ms (0.5 * T2)

Table 1. Sensitivities of NIST Probes Used for the NMR Interlaboratory Study

Magnet	Probe	^{1}H Sensitivity[1]	^{13}C Sensitivity[2]
600 MHz	Standard 5mm TXI	1,300	N.M.[3]
600 MHz	5mm TCI cryoprobe	7,285	1,339
900 MHz	5mm TCI cryoprobe	10,868	2,105

[1]using 0.1% ethylbeneze; [2]ASTM, 40% dioxane in C_6D_6;
[3]not measured

Table 2. Guidance on Experimental Time from S/N of 1st Fid of 2D Experiment, measured from NIST 600 MHz spectrometer with cold probe[1]

	$^{1}H,^{15}N$ gsHSQC			$^{1}H,^{13}C$ gsHSQC		
	Experimental Time	**1st Fid S/N**	**Experiment Number**	**Experimental Time**	**1st Fid S/N**	**Experiment Number**
SSS	2 h 0 min	61:1	1A	2 h 16 min	51:1	2C
Unlabeled NIST- Fab	N/A	N/A	N/A	18 h 13 min[3]	40:1	3A

[1]All experiments performed with a recycling delay = 1.0 s

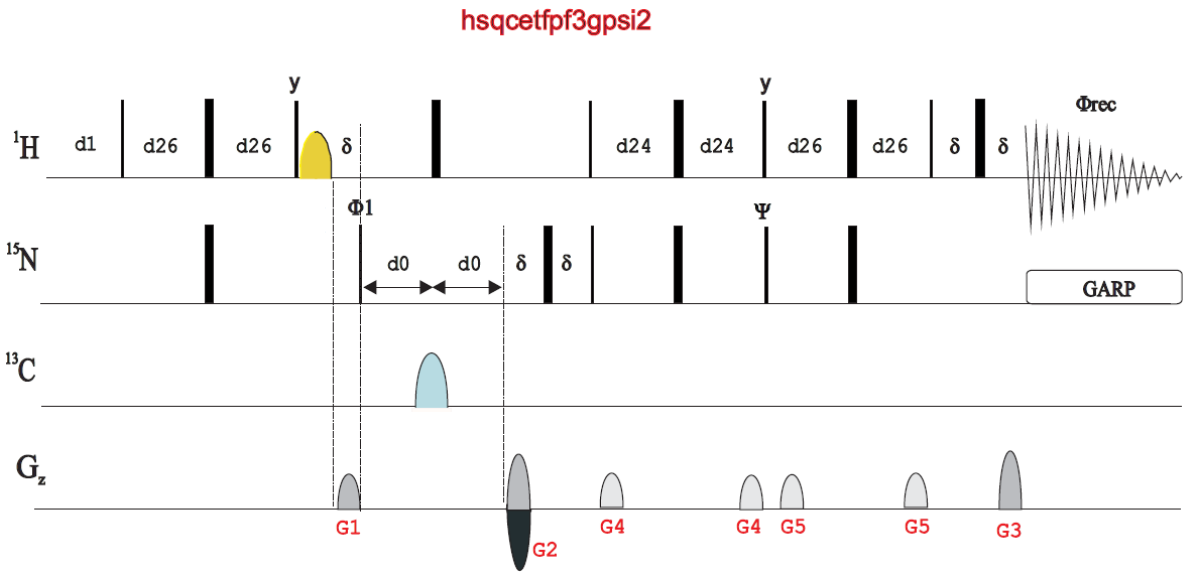

Figure 3. Schematic of the $^{1}H,^{15}N$ gsHSQC to be employed in the NMR interlaboratory study. The pulse scheme is taken from the Bruker Topspin 2.1 Pulse Program Library. For the purpose of this study, d24 = d26.

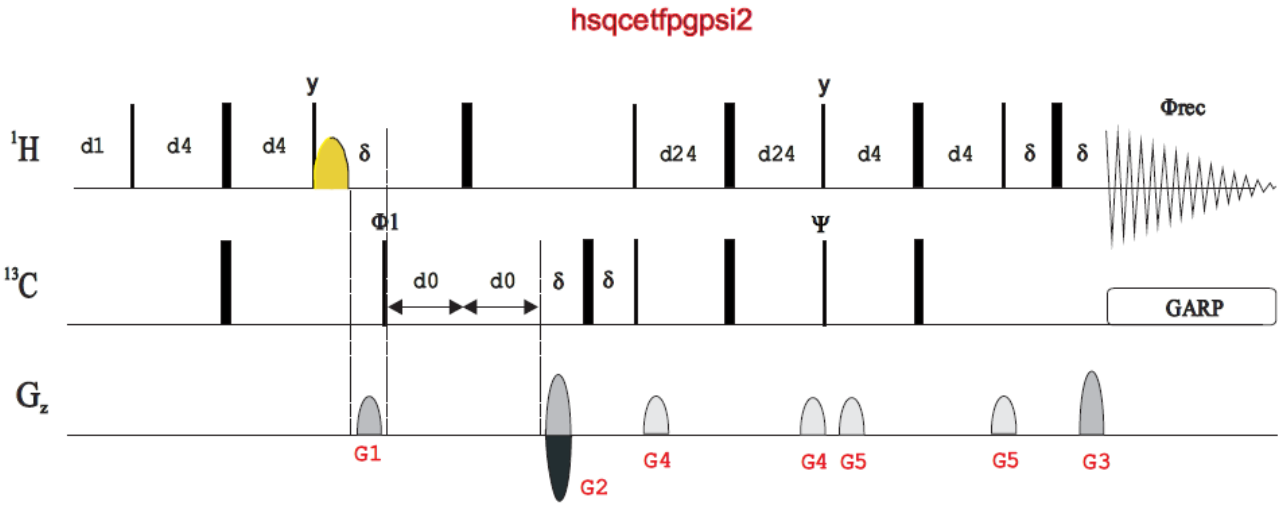

Figure 4. Schematic of the ^{1}H,^{13}C gsHSQC to be employed in the NMR interlaboratory study. The pulse scheme is taken from the Bruker Topspin 2.1 Pulse Program Library. For the purpose of this study, d24 = d4.

Table 3. Parameters for the ^{1}H,^{15}N gsHSQC Experiment

Scans per Increment	Collect enough scans per increment to achieve an average minimum S/N of 10:1
Recycling Delay	*Participant Discretion* Maximize S/N yet minimize overall experimental time
J^1_{NH}	93 Hz
^{1}H Carrier Position	Water resonance
^{1}H Sweep Width	20 ppm
^{1}H Acquisition time	100 ms
^{15}N Carrier Position	117 ppm
^{15}N Sweep Width	40 ppm
^{15}N Acquisition Time	20 ms (See Table 3 for number of total points)
Sampling Schedule	Uniform
Temperature	310 ± 0.1 K

Table 4. Varied Experimental Parameters for the $^{1}H,^{15}N$ gsHSQC

Field (MHz)	^{15}N Acquisition Time	^{15}N Total Points‡
500	20 ms	82
600	20 ms	98
700	20 ms	114
750	20 ms	122
800	20 ms	132
850	20 ms	138
900	20 ms	146

‡In this document, we refer to total number of points, e.g., 82 total points for the 500 MHz experiment would equate to 41 complex points.

Table 5. Experimental Parameters for $^{1}H,^{13}C$ gsHSQC

Scans per increment	***The overall goal is to achieve an average S/N of at least 10:1.*** ***U-^{15}N, 20%-^{13}C NIST-Fab*** **Exp 2A - 2D**: Same number of scans per increment **Exp 2E**: Twice the scans per increment of Experiment 2A **Unlabeled NIST-Fab** **Exp 3A:** Set scans per increment to achieve S/N of at least 10:1 **Exp 3B:** Same number of scans per increment as Exp. 3A
Recycling Delay	*Participant Discretion* Maximize S/N yet minimize overall experimental time
J^{1}_{CH}	145 Hz
^{1}H Carrier Position	Water resonance
^{1}H Sweep Width	14 ppm
^{1}H Acquisition time	100 ms
^{13}C Carrier Position	20 ppm
^{13}C Sweep Width	30 ppm
^{13}C Acquisition Time	*Varied, See Table 3*
Sampling Schedule	*Varied, See Table 3*
Temperature	310 ± 0.1 K

Table 6. Varied Experimental Parameters for $^{1}H,^{13}C$ gsHSQC

Sampling	Experiments 2A & 2B		Experiments 2C, 2D, 2E, 3A, 3B		
	Experiment 2A: Uniform **Experiment 2B:** 64_sched1.sched		**Experiment 3A**: Uniform **Experiments 2C,2D,2E,3B:** Use field dependent NUS schedule, below		
Field (MHz)	**^{13}C Acqu Time**	**^{13}C Total Points**	**^{13}C Acqu Time**	**^{13}C Total Points**	**NUS Schedule File**
500	17.0 ms	128†	25 ms	188	94_sched2_500MHz.sched
600	14.1 ms	128	25 ms	226	113_sched2_600MHz.sched
700	12.1 ms	128	25 ms	264	132_sched2_700MHz.sched
750	11.3 ms	128	25 ms	282	141_sched2_750MHz.sched
800	10.6 ms	128	25 ms	302	150_sched2_800MHz.sched
850	10.0 ms	128	25 ms	320	160_sched2_850MHz.sched
900	9.4 ms	128	25 ms	338	169_sched2_900MHz.sched

†Or 64 complex points.

E. Optional ^{1}H ,^{13}C and $^{1}H,^{15}N$ Experiments

The following experiments are optional and are not required. However, they would provide additional information on NUS and pulse sequence performance. They can be run on the system suitability sample or the unlabeled NIST-Fab.

1. Suggested $^{1}H,^{13}C$ Experiments

a. Generate your own NUS sampling schedule and re-run some of the experiments listed in Section D.

b. Run the same experiment at different temperatures. Please use any of the following: 15 °C, 25 °C, or 45 °C. *Remember to calibrate the temperature using the methanol-d4 sample!*

c. SOFAST-HMQC (Figure 5, Table 7)

2. Suggested $^{1}H,^{15}N$ Experiments

a. Run the same experiment at different temperatures. Please use any of the following: 15 °C, 25 °C, or 45 °C. *Remember to calibrate the temperature using the methanol-d4 sample!*

b. Phase sensitive HSQC: hsqcfpf3gpphwg (Figure 6)

c. SOFAST-HMQC: sfhmqcf3gpph (Figure 5, Table 7)

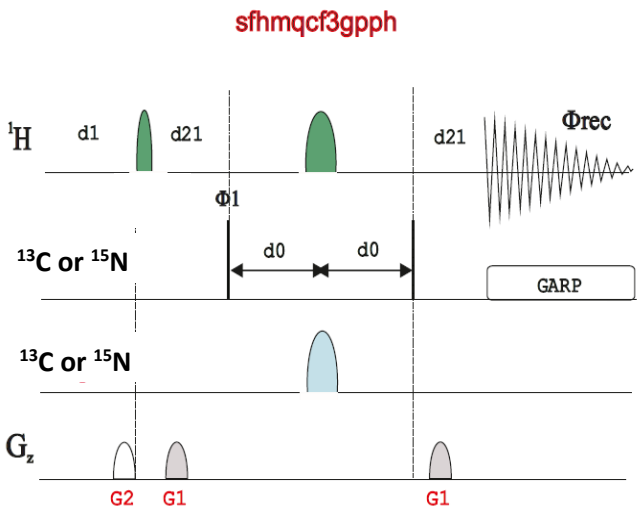

Figure 5. Schematic of the optional ^{1}H,X SOFAST-HMQC to be employed in the NMR interlaboratory study. The pulse scheme is taken from the Bruker Topspin 2.1 Pulse Program Library. Note that the default Bruker pulse sequence needs to be recoded for ^{13}C. Upon request, NIST can send the ^{13}C version for Bruker systems.

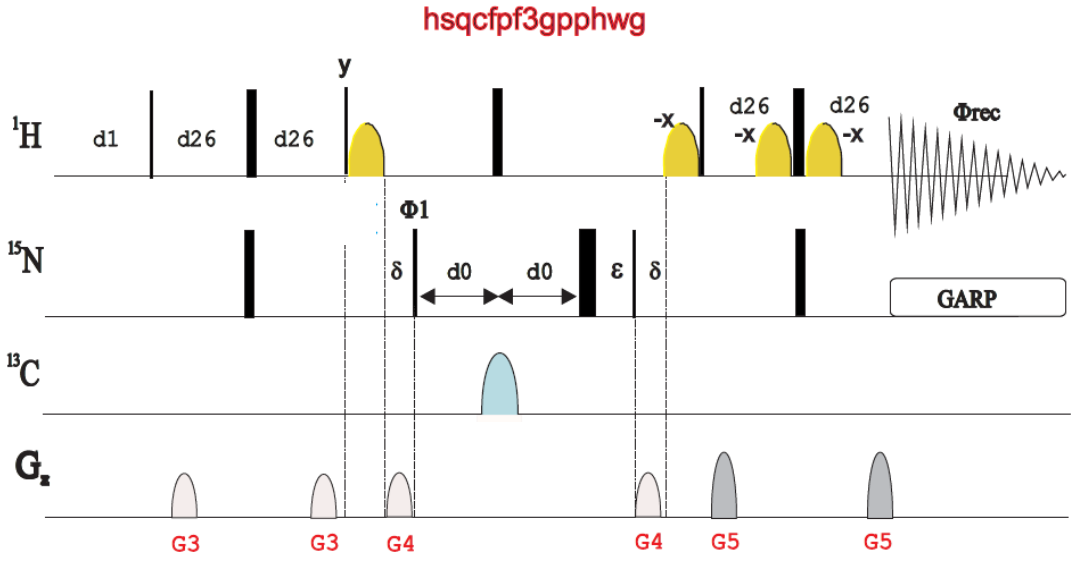

Figure 6. Schematic of the optional ^{1}H,^{15}N phase sensitive HSQC to be employed in the NMR interlaboratory study. The pulse scheme is taken from the Bruker Topspin 2.1 Pulse Program Library.

Table 7. Suggested Parameters for the Optional SOFAST-HMQC Experiments

	$^{1}H,^{15}N$ Experiment	$^{1}H,^{13}C$ Experiment
Acquisition time, t_2	50 ms[1]	50 ms[1]
Excitation Pulse	Pc.9.90[2]	Pc.9.90[2]
Refocusing Pulse	Reburp[3]	Reburp[3]
Excitation Window	5.55 ppm	4.44 ppm
Center of Shape Pulses	8.25 ppm	0.0 ppm

[1]Bruker hardware specifications limit acquisition time to 50 ms in t_2 for AVIII consoles.
[2]NIST observes minimal performance differences between the 90° PC.9 pulse and the Ernst Angle Optimized 120° PC.9 pulse.
[3]NIST has observed greatly enhanced performance using a Reburp refocusing pulse as compared against other refocusing pulses.

IV. Post-acquisition Handling of Data

This section will describe how to package all data and how to fill out all forms to be sent to NAPT.

A. Raw Data

1. Package all raw data and parameter files under a common directory tree, even if you collected the data on more than one instrument with different manufacturers.
2. Zip the data.

B. Master_V2.xls

Asides from your raw data, this is the most important file you will generate. It will be used by NAPT for data conversion to NMRPipe format. NIST will not receive this form.

Table 8 gives an example of this file. Please carefully fill out all fields. The file will be loaded on the NMR Study Online Forum. You may include as many datasets in the spreadsheet as needed as well as data from different instrument vendors.

data_id — *This is a hidden field in the XLS-formatted form. Each dataset in the table will become populated with spec001, spec002, spec003, etc.*

datafile_name — *Please list the directory tree for the vendor-formatted raw data. The file path is relative and needs to contain text with no spaces. Filename in file path will be "ser" "fid" or have the extensions "*.jdf" or "*.JDF."*

Examples:
data/mab1/hmqc/3/ser
agilent/mab2/hsqc.fid/fid
study/buffer/ghn.jdf

sample_info — *Please list the sample (e.g., unlabeled NIST-Fab or U-^{15}N, 20%-^{13}C NIST-Fab) and experiment performed (e.g., Exp. 1A, 2A). If you are running optional experiments, please list the pulse sequence used and soft pulse parameters, if SOFAST data was collected. This field must not be blank. Spaces are allowed.*

Table 8. Example of master_V2.xls. The EXCEL version of the file is located on the NMR study online forum. See main text for description of each column.

datafile_name	sample_info	gradient_enhanced	x_car_ppm	y_nucleus	y_sw_hz	y_obs_mhz	y_car_ppm	temperature_k	scans	nus_name
NIST/CH_gHSQC_US/5/ser	**SSS**, Exp. 2A	yes	4.721	13C	7692.308	226.351	25.000	310.0	128	
NIST/CH_gHSQC_n50/7/ser	**SSS**, Exp. 2B	yes	4.721	13C	7692.308	226.351	25.000	310.0	128	nuslist

Shorthand Key (for sample info):

SSS:	system suitability sample
NIST-Fab:	unlabeled NIST-Fab derived from NISTmAb
Exp. 2A:	Experiment 2A. For required experiments, just list experiment number
Exp. E2B	Optional Experimental in Section E, exp. 2B
Sched. 1	NUS schedule 1
Sched. 2	NUS schedule 2
Sched. X	custom NUS schedule

gradient_enhanced	*Choose "yes"* or *"no" from the dropdown menu.*
x_car_ppm	*^{1}H transmitter position in ppm. No commas or spaces. This field may be left blank.*
y_nucleus	*Choose "^{13}C" or "^{15}N" from the dropdown menu.*
y_sw_hz	*Total ^{13}C or ^{15}N sweep width in Hz. This is a floating point value between 500 and 50 000.0. No commas or spaces. This field may be left blank.*
y_obs_mhz	*Observe frequency of ^{13}C or ^{15}N in MHz. This is a floating point value between 10 and 14 000.0. No commas or spaces. This field may be left blank.*
y_car_ppm	*^{13}C or ^{15}N carrier position in ppm. This is a floating point value between -10.0 and 200.0. No commas or spaces. This field may be left blank.*
temperature_k	*Temperature of experiment in Kelvin. This is a floating point value between 250.0 and 350.0. No commas or spaces. This field may be left blank.*
scans	*Number of scans per increment. This field may be left blank.*
nus_name	*name of NUS schedule file. Please leave blank if uniform sampling (US) was used.* In the data directory, the following files may be present if NUS acquisition was performed: "nuslist" "vclist" "scheduler" "*.sch".

C. Form A: Instrumentation Survey V2.docx. The purpose of this survey is to catalog the field strengths used in this interlaboratory study. NAPT will have the form available to be downloaded in Word format.

An example of Form A is given below:

Form A: The Instrumentation Survey

Please provide the following information about your NMR system. The information provided here will not be used for discussions of head-to-head performance comparisons (*e.g.*, brand-name comparisons).

Please submit this form directly to NAPT, who will compile the information and send NIST a complete list of magnets, consoles, and probes used in the study.

Here are is a NIST example:

Magnet #1

Frequency of ^{1}H	900 MHz
Manufacturer	Bruker BioSpin
Console	AVANCE III
Probe (e.g., cold or RT probe; double or triple resonance; pulsed gradient capability)	5mm TCI ^{1}H-^{13}C/^{15}N/D Cryoprobe™, Z-gradients

Please add as many magnets as you used for the study.

Magnet #1

Frequency of ^{1}H	
Manufacturer	
Console	
Probe (e.g., cold or RT probe; double or triple resonance; pulsed gradient capability)	

D. Form B: Authorship and Acknowledgment Form.docx The Authorship and Acknowledgement Form is posted for download on the NAPT website. The contributing scientists are authors of the final manuscript. This list should be in compliance with the guidelines of your institution for defining authorship. An author is generally considered to be an individual who has made substantial intellectual contributions to a scientific investigation. All authors should meet the following three criteria, and all those who meet the criteria should be authors:[5]

1. Scholarship: Contribute significantly to the conception, design, execution, and/or analysis and interpretation of data.
2. Authorship: Participate in drafting, reviewing, and/or revising the manuscript for intellectual content.
3. Approval: Approve the manuscript to be published.

NIST has structured this interlaboratory study such that one or more scientists at each participating institution will meet these criteria predominantly through the analysis and interpretation of data, by reviewing/revising the manuscript, and by approving the manuscript. In addition to a work email address, the Authorship and Acknowledgement Form asks each author to provide a relatively permanent (personal) email address, such as a Gmail, Yahoo, or Hotmail account. This address will be used only when a work address fails, and we need to obtain the scientist's signature on a copyright agreement. The address of each author must correspond to that of his/her employer at the time the measurements were performed. If the author has changed employer, please provide the new address, too, since new addresses may be noted in the submitted manuscript.

An example of Form B is given below:

Form B: Authorship and Acknowledgement Form

Please provide a list of authors in the form below. Add rows, as necessary.

Please add your acknowledgements. Please understand that these acknowledgements will be edited to conform to the policies of the journal.

NAPT will release the author list to NIST after all anonymized data has been given to NIST.

Name	**Institution, Department , Address**	**Institutional E-Mail/ Personal email**	**Contribution to Study** (Succinct description please)
Acknowledgements: **1)**			

D. Uploading of Data and Forms to Microsoft OneDrive

NAPT will be creating individual accounts for each participant through Microsoft OneDrive. You will receive information for this site directly from NAPT. NIST will not have access to your online folder.

NAPT will be converting all data to NMRPipe format using a conversion tool provided to NAPT by Frank Delaglio of NIST. After data conversion, NAPT will rename all data with a random anonymized code. NAPT will provide the anonymized data to NIST. In addition, each institution will receive back their anonymized data so it can be traced throughout the data analysis.

V. Procedures for Communication with NIST and NAPT

During this interlaboratory study participants may communicate by email with NIST scientists at any time for clarification of measurement procedures; to report problems with the NAPT website; general suggestions, comments, and revisions regarding the manuscript; etc.

NMR Interlaboratory Study Online Forum. An online forum has been developed with the following objectives:

1) Repository of study documentation, administrative forms (Form A and Form B), NUS schedules, all vendor-specific pulse sequence codes, Topspin 2.x NUS pulse sequence.
2) Technical Discussion.

Additional directions will be sent out via email, and terms of usage on listed in the "Welcome to the Study" page in the Online Forum.

Specific questions or problems after analysis of your data: In order to maintain anonymity of data, specific questions regarding the analysis of your data should be done through NAPT. NAPT will remove identifiable information from your inquiry and send it anonymously to NIST. NIST will send its answer to NAPT who will forward it to the appropriate institution.

Processing your Data. *The blinded data are optimized for processing with the software NMRPipe.* Other software will likely be *unable* to process the blinded data.

NAPT will contact you with reporting instructions, the website URL, and e-mail addresses.

APPENDIX I. Guidance for Determination of S/N for 1st FID of 2D Experiment

For Sample # 2, U-^{15}N, 20% ^{13}C labeled NIST Fab System Suitability Standard (SSS):

1. Collect and Fourier Transform 1st FID of gsHSQC experiment and calculate S/N using the regions defined in Table 9. The target for the ^{1}H-^{15}N gsHSQC is 60:1 and for the ^{1}H-^{13}C gsHSQC is 40:1.
2. Collect full 2D spectrum and calculate S/N from the average of peaks heights in the signal region (set threshold just above noise) by the RMS amplitude of noise peak heights in the noise region (set threshold so noise peaks cover most of noise region) to ensure a minimum S/N of 10:1.

For Sample #3, unlabeled NIST Fab

1. For ^{1}H-^{13}C gsHSQC experiment we find the unlabeled NIST requires approximately 8x the number of scans as the SSS to achieve comparable S/N.
2. Collect and Fourier Transform 1st FID of the ^{1}H-^{13}C gsHSQC and calculate S/N using the regions defined in Table 9, the target is 40:1.
3. Collect full 2D spectrum and calculate S/N from the average of peaks heights in the signal region (set threshold just above noise) by the RMS amplitude of noise peak heights in the noise region (set threshold so noise peaks cover most of noise region) to ensure a minimum S/N of 10:1.

Table 8: Signal and Noise Regions[1]

Experiment	Dimensionality	Signal Region	Noise Region
^{1}H-^{15}N gsHSQC (SSS only)	1D	^{1}H: 6.5 10.5 ppm	^{1}H: -4.0 – 1.0 ppm
	2D	^{1}H: 5.5-13.0 ppm	^{1}H: -4.0 – 1.0 ppm
		^{15}N: 104 – 135 ppm	^{15}N: 110 – 125 ppm
^{1}H-^{13}C gsHSQC	1D	^{1}H: -0.5 – 2.5 ppm	^{1}H: 8.75 – 11.25 ppm[2]
	2D	^{1}H: -0.5 – 2.5 ppm	^{1}H: 8.75 – 11.25 ppm[2]
		^{13}C: 8.5 – 27.5 ppm	^{13}C: 15 – 30 ppm

[1]Guidance is for online calculations of S/N on the spectrometer console. Example calculations were performed in Topspin 3.0 using the '.sino' and 'pp' commands for 1D and 2D calculations respectively. Further studies are underway to ensure conformity of Agilent and JEOL platforms.
[2]Two signals appeared between 6.0 and 8.0 ppm. The noise region therefore had to be shifted further downfield.

APPENDIX I. NUS Set-up on Topspin 2.x

Please email Robert Brinson (robert.brinson@nist.gov) for the pulse program.

1. Import pulse program, *AVI_hsqcetfpgpsi2_nus.txt*, into your pulse program folder. Typically, this directory tree is similar to this: topspin/exp/stan/nmr/lists/pp/user.

2. Import relevant NUS schedules into the vc folder. Typically, this is located at topspin/exp/stan/nmr/lists/vc.

3. Under Acquistion parameters tab (AcquPars), set the following:

PULPROG	AVI_hsqcetfpgpsi2_nus.txt
FnMODE	undefined

4. Set up all other experimental parameters as normal.

5. Pulse program testing with uniform sampling (US) acquisition.

Import an acquisition schedule that contains every point into the vc folder. For 64 complex points, number the points 0 through 63. If you need help with this testing, please contact Robert Brinson. *Note: The first increment must be 0!*

APPENDIX II. MSDS Sheets.

DATE: 04 MAY 2016

Product Identifier

RM Name: NIST-Fab derived from NISTmAb [Candidate RM 8670 (Lot #3F1b)].

Under the U.S. Department of Labor, Occupational Safety and Health Administration (OSHA) 29 CFR 1910.1200, this Reference Material (RM) is NOT classified as a physical hazard or a health hazard, a simple asphyxiant, combustible dust, pyrophoric gas, or hazard not otherwise classified. There are no hazard pictograms, hazard statements, or signal word associated with it. Material Safety Data Sheet information is not required. This document may be used in conjunction with your hazard communication program.

Description: This candidate reference material (RM) is intended for the critical evaluation of the experimental practices of nuclear magnetic resonance spectroscopy (NMR). The RM is intended for a variety of uses involving NMR. These include, but are not necessarily limited to: precision tests, system suitability tests, establishing method or instrument performance and variability, comparing changing analytical test methods, assisting in method qualification, etc. A unit of NIST-Fab consists of 0.3 mL of solution containing up to 16 mg NIST-Fab protein per sample in 25 mmol/L bis-tris-d19 buffer, pH 6.0, in 95% H_2O and 5% D_2O.

Unlabeled NIST-Fab was enzymatically produced from Candidate RM 8670 (Lot #3F1b), an IgG1κ, by application of immobilized papain cross-linked on agarose beaded support. NIST-Fab has been extensively tested for purity by SDS-PAGE in reducing and nonreducing conditions, by ESI-MS, and by NMR. The test results show that NIST-Fab comprises a single (47548 ± 30) Da protein. NIST-Fab contains no glycosylation.

U-^{15}N, 20%-^{13}C NIST-Fab was produced in *P. pastoris* and analyzed in a similar fashion to the unlabeled NIST-Fab.

Additional Notes: NIST-Fab IS INTENDED FOR LABORATORY USE ONLY. NIST-Fab IS NOT INTENDED FOR ANIMAL OR HUMAN CONSUMPTION nor is it intended for any form of CLINICAL TESTING OR USE IN ANY WAY. As a general rule, personal protective equipment should always be worn during any laboratory procedure. This includes, but is not limited to, safety glasses, gloves, and a laboratory coat. This RM should be stored at 4 °C.

Disposal: NIST-Fab from RM 8670 should be disposed of in accordance with local, state, and federal regulations.

Transport Information: This material is not regulated by the U.S. Department of Transportation (DOT) and/or International Air Transportation Association (IATA).

MATERIAL SAFETY DATA SHEET

1 CHEMICAL PRODUCT AND COMPANY INFORMATION

Product Description BIS-TRIS (D19, 98%)

Cambridge Isotope Laboratories, Inc.
50 Frontage Rd
ANDOVER, MA 01810
USA

E-mail cilsales@isotope.com
Web Site www.isotope.com

Phone Numbers
Emergency Contact Chemtrec
Emergency Phone 1-800-424-9300 (24 hours)

Customer Service Phone 1-800-322-1174 (8:30-5:30 EST)
Transportation 1-202-483-7616 (24 hours)
General Use Additional synonyms: Bis(2-hydroxyethyl)amino-tris(hydroxymethyl)methane
2,2-Bis(hydroxymethyl)-2,2',2"-nitrilotriethanol

2 HAZARDS IDENTIFICATION

OSHA Hazards
Irritant

GHS Classification
Skin irritation (Category 2)
Eye irritation (Category 2A)
Specific Target Organ Toxicity - Single exposure (Category 3)

GHS Label Information

Signal Word Warning

Hazards Identification
Exclamation mark

Hazard Statement(s)
Causes skin irritation.
Causes serious eye irritation.

MATERIAL SAFETY DATA SHEET

May cause respiratory irritation.

Precautionary Statement(s)

Avoid breathing dust/fume/gas/mist/vapours/spray.
Wash skin thoroughly after handling.
Use only outdoors or in a well-ventilated area.
Wear protective gloves/protective clothing/eye protection/face protection.
IF ON SKIN: Wash with soap and water.
IF INHALED: Remove victim to fresh air. Keep at rest in a comfortable position.
IF IN EYES: Rinse with water for several minutes. Remove contact lenses.
If skin irritation occurs: Get medical advice/attention.

HMIS Ratings

Physical	0
Flammability	0
Health	2

NFPA Codes

Fire	0
Health	2
Reactivity	0

Potential Health Effects

Eyes	Causes eye irritation.
Ingestion	May be harmful if swallowed.
Inhalation	May be harmful if inhaled. Causes respiratory tract irritation.
Skin	May be harmful if absorbed through the skin. Causes skin irritation.

3 COMPOSITION / INFORMATION ON INGREDIENTS

Composition / Information on Ingredients

Synonyms	Bis(2-hydroxyethyl)amino-tris(hydroxymethyl)methane
Chemical Formula	(DOCD2)3C-N(CD2CD2OD)2
Molecular Weight	228.36
CAS No.	352534-93-1
EC No.	230-237-7

4 FIRST AID MEASURES

First Aid Measures

Eyes	Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.
Ingestion	Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

MATERIAL SAFETY DATA SHEET

Inhalation	If breathed in, move person to fresh air. If not breathing, give artificial respiration. Consult a physician.
Skin	Wash with soap and plenty of water. Consult a physician.
Additional Information	Consult a physician. Show this safety data sheet to the doctor in attendance. Move out of dangerous area.

5 FIRE FIGHTING MEASURE

Fire Fighting Measures

Extinguishing Media	Use water spray, alcohol-resistant foam, dry chemical, or carbon dioxide.
Fire Fighting Equipment	Wear self contained breathing apparatus for fire fighting if necessary.

6 ACCIDENTAL RELEASE MEASURES

Accidental Release Measures

Personal Precautions	Use personal protective equipment. Avoid dust formation. Avoid breathing vapors, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas. Avoid breathing dust.
Environmental Precautions	Do not empty into drains.
Methods and materials for containment and cleanup	Pick up and arrange disposal without creating dust. Sweep up and shovel. Keep in suitable, closed containers for disposal.

7 HANDLING AND STORAGE

Precautions for Safe Handling

Handling	Avoid contact with skin and eyes. Avoid formation of dust and aerosols. Provide appropriate exhaust ventilation at places where dust is formed. Normal measures for preventive fire protection.

Conditions for Safe Storage

Storage	Store at room temperature away from light and moisture.

MATERIAL SAFETY DATA SHEET

8 EXPOSURE CONTROL / PERSONAL PROTECTION

Personal Protecton

Eyes - Face	Wear safety glasses with side shields (or goggles) and a face shield.
Skin	Choose body protection according to the amount and concentration of the dangerous substance at the work place.
Respiratory	Wear appropriate NIOSH/MSHA approved respirator.
Protective Clothing	Wear suitable protective clothing and gloves.
Work Hygienic Practices	Handle in accordance with good industrial hygiene and safety practice. Wash hands before breaks and at the end of workday.

9 PHYSICAL AND CHEMICAL PROPERTIES

Physical and Chemical Properties (Unlabeled Compound)

Form	Powder
Color	White
Odor	No data available
Odor Threshold	No data available
pH	9.5 - 11.0 at 209.2 g/l at 25 °C (77 °F)
Melting Point	102 - 103 °C (216 - 217 °F)
Boiling Point	No data available
Flashpoint	No data available
Evaporation Rate	No data available
Lower Explosion Limit	No data available
Upper Explosion Limit	No data available
Vapor Pressure	No data available
Vapor Density	No data available
Density	No data available
Solubility in Water	ca.209.2 g/l at 20 °C (68 °F)
Partition Coefficient	No data available
Auto Ignition Temperature	No data available

MATERIAL SAFETY DATA SHEET

10 STABILITY AND REACTIVITY

Stability and Reactivity

Chemical Stability	Two years after receipt if stored as stated in "Storage" section. Re-QC after 2 years.
Conditions to Avoid	Not available
Hazardous decomposition products	Formed under fire conditions: Carbon oxides, nitrogen oxides.
Materials to avoid	Strong oxidizing agents

11 TOXICOLOGICAL INFORMATION

Acute Toxicity

Serious Damage/Eye Irritation	No data available
Skin Corrosion/Irritation	No data available
Inhalation	No data available
Respiratory or Skin Sensitization	Not available
Germ Cell Mutagenicity	Not available
IARC	No component of this product present at levels greater than or equal to 0.1% is identifiable as probable, possible, or confirmed human carcinogen by IARC.
ACGIH	No component of this product present at levels greater than or equal to 0.1% is identifiable as a carcinogen or potential carcinogen by ACGIH.
NTP	No component of this product present at levels greater than or equal to 0.1% is identifiable as a known or anticipated carcinogen by NTP.
OSHA	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.
Reproductive Toxicity	Not available

Specific Target Organ Toxicity

MATERIAL SAFETY DATA SHEET

Single Exposure	Inhalation - May cause respiratory irritation.
Repeated Exposure	No data available

Signs and Symptoms of Exposure

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

Other Information

RTECS	No data available

12 ECOLOGICAL INFORMATION

Toxicity

Persistance and Degradability	Not available
Bioaccumulative Potential	Not available
Mobility in Soil	Not available
PBT and vPvB Assessment	Not available
Other Adverse Effects	Not available

13 DISPOSAL CONSIDERATIONS

Disposal Considerations

Product Disposal	Waste materials should be disposed of under conditions which meet Federal, State, and Local environmental control regulations.

14 TRANSPORT INFORMATION

Transportation Information-DOT/IATA/IMDG

Special Shipping Notes	Not dangerous goods.

MATERIAL SAFETY DATA SHEET

15 REGULATORY INFORMATION

Regulatory Information

Irritant.

SARA

Sara 302 Component	No chemicals in this material are subject to the reporting requirements.
Sara 313 Component	No chemicals in this material are subject to the reporting requirements.

SARA 311/312 Hazards

Acute (Y/N)	Y

STATE REGULATIONS

Massachusetts Right to Know	No
Pennsylvania Right to Know	Yes
New Jersey Right to Know	Yes
California Proposition 65	This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

Canada

DSL Status	All components are on the Canadian DSL list.

EU

Hazard	Irritant

Safety Statements

Do not breathe dust.
After contact with skin, wash immediately with plenty of water.
In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.
After contact with skin, wash immediately with plenty of water.
Wear suitable protective clothing, gloves and eye/face protection.
In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
Use only in well-ventilated areas.
In case of accident by inhalation: remove casualty to fresh air and keep at rest.

Risk Statements

Wear suitable protective clothing, gloves and eye/face protection.

MATERIAL SAFETY DATA SHEET

16 OTHER INFORMATION

Additional MSDS Information	This product is not radioactive. The data given for this product are those of the corresponding unlabeled compound, unless specifically indicated otherwise. Health and safety data for labeled compounds are generally not available, but are assumed to be similar or identical to the corresponding unlabeled compound.
Approved On	11/25/13
Version Number	2

SAFETY DATA SHEET

Version 5.4
Revision Date 07/31/2014
Print Date 12/01/2015

1. PRODUCT AND COMPANY IDENTIFICATION

1.1 Product identifiers

Product name : Methanol-d4 solution

Product Number : 721948
Brand : Aldrich

CAS-No. : 811-98-3

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich
3050 Spruce Street
SAINT LOUIS MO 63103
USA

Telephone : +1 800-325-5832
Fax : +1 800-325-5052

1.4 Emergency telephone number

Emergency Phone # : (314) 776-6555

2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)
Flammable liquids (Category 2), H225
Acute toxicity, Oral (Category 3), H301
Acute toxicity, Inhalation (Category 3), H331
Acute toxicity, Dermal (Category 3), H311
Skin irritation (Category 2), H315
Eye irritation (Category 2A), H319
Specific target organ toxicity - single exposure (Category 1), H370

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 GHS Label elements, including precautionary statements

Pictogram

Signal word Danger

Hazard statement(s)
H225 Highly flammable liquid and vapour.
H301 + H311 + H331 Toxic if swallowed, in contact with skin or if inhaled
H315 Causes skin irritation.
H319 Causes serious eye irritation.
H370 Causes damage to organs.

Precautionary statement(s)
P210 Keep away from heat/sparks/open flames/hot surfaces. - No smoking.
P233 Keep container tightly closed.

P240	Ground/bond container and receiving equipment.
P241	Use explosion-proof electrical/ ventilating/ lighting/ equipment.
P242	Use only non-sparking tools.
P243	Take precautionary measures against static discharge.
P260	Do not breathe dust/ fume/ gas/ mist/ vapours/ spray.
P264	Wash skin thoroughly after handling.
P270	Do not eat, drink or smoke when using this product.
P271	Use only outdoors or in a well-ventilated area.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301 + P310	IF SWALLOWED: Immediately call a POISON CENTER or doctor/ physician.
P303 + P361 + P353	IF ON SKIN (or hair): Remove/ Take off immediately all contaminated clothing. Rinse skin with water/ shower.
P304 + P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P307 + P311	IF exposed: Call a POISON CENTER or doctor/ physician.
P322	Specific measures (see supplemental first aid instructions on this label).
P330	Rinse mouth.
P332 + P313	If skin irritation occurs: Get medical advice/ attention.
P337 + P313	If eye irritation persists: Get medical advice/ attention.
P361	Remove/Take off immediately all contaminated clothing.
P370 + P378	In case of fire: Use dry sand, dry chemical or alcohol-resistant foam for extinction.
P403 + P233	Store in a well-ventilated place. Keep container tightly closed.
P403 + P235	Store in a well-ventilated place. Keep cool.
P405	Store locked up.
P501	Dispose of contents/ container to an approved waste disposal plant.

2.3 Hazards not otherwise classified (HNOC) or not covered by GHS - none

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.1 Substances

Formula	:	CD_4O
Molecular Weight	:	36.07 g/mol
CAS-No.	:	811-98-3
EC-No.	:	212-378-6

Hazardous components

Component	Classification	Concentration
(2H4)Methanol		
	Flam. Liq. 2; Acute Tox. 3; Skin Irrit. 2; Eye Irrit. 2A; STOT SE 1; H225, H301 + H311 + H331, H315, H319, H370	90 - 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

4. FIRST AID MEASURES

4.1 Description of first aid measures

General advice
Consult a physician. Show this safety data sheet to the doctor in attendance.Move out of dangerous area.

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.

In case of skin contact
Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician.

In case of eye contact
Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.

If swallowed
Do NOT induce vomiting. Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
no data available

5. FIREFIGHTING MEASURES

5.1 Extinguishing media

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture
no data available

5.3 Advice for firefighters
Wear self contained breathing apparatus for fire fighting if necessary.

5.4 Further information
Use water spray to cool unopened containers.

6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures
Wear respiratory protection. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Remove all sources of ignition. Evacuate personnel to safe areas. Beware of vapours accumulating to form explosive concentrations. Vapours can accumulate in low areas.
For personal protection see section 8.

6.2 Environmental precautions
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up
Contain spillage, and then collect with an electrically protected vacuum cleaner or by wet-brushing and place in container for disposal according to local regulations (see section 13).

6.4 Reference to other sections
For disposal see section 13.

7. HANDLING AND STORAGE

7.1 Precautions for safe handling
Avoid contact with skin and eyes. Avoid inhalation of vapour or mist.
Use explosion-proof equipment.Keep away from sources of ignition - No smoking.Take measures to prevent the build up of electrostatic charge.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities
Keep container tightly closed in a dry and well-ventilated place. Containers which are opened must be carefully resealed and kept upright to prevent leakage.

Moisture sensitive.

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters

Components with workplace control parameters

Component	CAS-No.	Value	Control parameters	Basis
(2H4)Methanol	811-98-3	TWA	200 ppm 260 mg/m3	USA. Occupational Exposure Limits (OSHA) - Table Z-1 Limits for Air Contaminants
	Remarks	The value in mg/m3 is approximate.		
		TWA	200 ppm	USA. ACGIH Threshold Limit Values (TLV)
		Headache Eye damage Substances for which there is a Biological Exposure Index or Indices (see BEI® section) Danger of cutaneous absorption		
		STEL	250 ppm	USA. ACGIH Threshold Limit Values (TLV)
		Headache Eye damage Substances for which there is a Biological Exposure Index or Indices (see BEI® section) Danger of cutaneous absorption		
		TWA	200 ppm 260 mg/m3	USA. OSHA - TABLE Z-1 Limits for Air Contaminants - 1910.1000
		Skin notation		
		STEL	250 ppm 325 mg/m3	USA. OSHA - TABLE Z-1 Limits for Air Contaminants - 1910.1000
		Skin notation		
		TWA	200 ppm 260 mg/m3	USA. NIOSH Recommended Exposure Limits
		Potential for dermal absorption		
		ST	250 ppm 325 mg/m3	USA. NIOSH Recommended Exposure Limits
		Potential for dermal absorption		

Biological occupational exposure limits

Component	CAS-No.	Parameters	Value	Biological specimen	Basis
(2H4)Methanol	811-98-3	Methanol	15 mg/l	In urine	ACGIH - Biological Exposure Indices (BEI)
	Remarks	End of shift (As soon as possible after exposure ceases)			

8.2 Exposure controls

Appropriate engineering controls
Avoid contact with skin, eyes and clothing. Wash hands before breaks and immediately after handling the product.

Personal protective equipment

Eye/face protection
Face shield and safety glasses Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Full contact
Material: butyl-rubber
Minimum layer thickness: 0.3 mm
Break through time: 480 min
Material tested:Butoject® (KCL 897 / Aldrich Z677647, Size M)

Splash contact
Material: Nitrile rubber
Minimum layer thickness: 0.4 mm
Break through time: 30 min
Material tested:Camatril® (KCL 730 / Aldrich Z677442, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374
If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

Body Protection
Complete suit protecting against chemicals, Flame retardant antistatic protective clothing, The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection
Where risk assessment shows air-purifying respirators are appropriate use a full-face respirator with multi-purpose combination (US) or type AXBEK (EN 14387) respirator cartridges as a backup to engineering controls. If the respirator is the sole means of protection, use a full-face supplied air respirator. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure
Prevent further leakage or spillage if safe to do so. Do not let product enter drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: liquid
b)	Odour	no data available
c)	Odour Threshold	no data available
d)	pH	no data available
e)	Melting point/freezing point	no data available
f)	Initial boiling point and boiling range	65.4 °C (149.7 °F) at 1,013 hPa (760 mmHg)
g)	Flash point	11 °C (52 °F) - closed cup
h)	Evapouration rate	no data available
i)	Flammability (solid, gas)	no data available
j)	Upper/lower flammability or explosive limits	Upper explosion limit: 36 %(V) Lower explosion limit: 6 %(V)
k)	Vapour pressure	546.6 hPa (410.0 mmHg) at 50.0 °C (122.0 °F)
l)	Vapour density	no data available
m)	Relative density	0.888 g/cm3
n)	Water solubility	no data available
o)	Partition coefficient: n-octanol/water	no data available
p)	Auto-ignition temperature	no data available
q)	Decomposition temperature	no data available

r)	Viscosity	no data available
s)	Explosive properties	no data available
t)	Oxidizing properties	no data available

9.2 Other safety information
no data available

10. STABILITY AND REACTIVITY

10.1 Reactivity
no data available

10.2 Chemical stability
Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions
Vapours may form explosive mixture with air.

10.4 Conditions to avoid
Avoid moisture.
Heat, flames and sparks. Extremes of temperature and direct sunlight.

10.5 Incompatible materials
acids, Acid chlorides, Acid anhydrides, Oxidizing agents, Alkali metals, Reducing agents

10.6 Hazardous decomposition products
Other decomposition products - no data available
Hazardous decomposition products formed under fire conditions. - Carbon oxides
In the event of fire: see section 5

11. TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Acute toxicity
LD50 Oral - rat - 5,628 mg/kg

LC50 Inhalation - rat - 4 h - 64000 ppm

LD50 Dermal - rabbit - 15,800 mg/kg

no data available

Skin corrosion/irritation
Skin - rabbit
Result: Skin irritation - 24 h

Serious eye damage/eye irritation
Eyes - rabbit
Result: Eye irritation

Respiratory or skin sensitisation
no data available

Germ cell mutagenicity
no data available

Carcinogenicity

IARC:	No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.
ACGIH:	No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by ACGIH.
NTP:	No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.
OSHA:	No component of this product present at levels greater than or equal to 0.1% is identified as a

carcinogen or potential carcinogen by OSHA.

Reproductive toxicity
no data available

no data available

Specific target organ toxicity - single exposure
Causes damage to organs.

Specific target organ toxicity - repeated exposure
no data available

Aspiration hazard
no data available

Additional Information
RTECS: PC1400000

Weakness, Confusion., Drowsiness, Unconsciousness, May cause convulsions., Dizziness, Gastrointestinal disturbance, Nausea, Headache, Vomiting, Warning: contains methanol. May be fatal or cause blindness if swallowed. Cannot be made nonpoisonous.

Liver - Irregularities - Based on Human Evidence
Liver - Irregularities - Based on Human Evidence

12. ECOLOGICAL INFORMATION

12.1 Toxicity

Toxicity to fish	LC50 - Oncorhynchus mykiss (rainbow trout) - 19,000 mg/l - 96 h
Toxicity to daphnia and other aquatic invertebrates	EC50 - Daphnia magna (Water flea) - 24,500 mg/l - 48 h

12.2 Persistence and degradability
no data available

12.3 Bioaccumulative potential
no data available

12.4 Mobility in soil
no data available

12.5 Results of PBT and vPvB assessment
PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

12.6 Other adverse effects

no data available

13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods

Product
Burn in a chemical incinerator equipped with an afterburner and scrubber but exert extra care in igniting as this material is highly flammable. Offer surplus and non-recyclable solutions to a licensed disposal company. Contact a licensed professional waste disposal service to dispose of this material.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
UN number: 1230 Class: 3 Packing group: II
Proper shipping name: Methanol
Reportable Quantity (RQ): 5000 lbs
Marine pollutant: No

Poison Inhalation Hazard: No

IMDG
UN number: 1230 Class: 3 (6.1) Packing group: II EMS-No: F-E, S-D
Proper shipping name: METHANOL
Marine pollutant: No

IATA
UN number: 1230 Class: 3 (6.1) Packing group: II
Proper shipping name: Methanol

15. REGULATORY INFORMATION

SARA 302 Components
SARA 302: No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components
The following components are subject to reporting levels established by SARA Title III, Section 313:

	CAS-No.	Revision Date
(2H4)Methanol	811-98-3	1993-04-24

SARA 311/312 Hazards
Fire Hazard, Acute Health Hazard, Chronic Health Hazard

Massachusetts Right To Know Components

	CAS-No.	Revision Date
(2H4)Methanol	811-98-3	1993-04-24

Pennsylvania Right To Know Components

	CAS-No.	Revision Date
(2H4)Methanol	811-98-3	1993-04-24

New Jersey Right To Know Components

	CAS-No.	Revision Date
(2H4)Methanol	811-98-3	1993-04-24

California Prop. 65 Components
This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

16. OTHER INFORMATION

Full text of H-Statements referred to under sections 2 and 3.

Acute Tox.	Acute toxicity
Eye Irrit.	Eye irritation
Flam. Liq.	Flammable liquids
H225	Highly flammable liquid and vapour.
H301	Toxic if swallowed.
H301 + H311 + H331	Toxic if swallowed, in contact with skin or if inhaled
H311	Toxic in contact with skin.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H331	Toxic if inhaled.

HMIS Rating

Health hazard:	2
Chronic Health Hazard:	*
Flammability:	3
Physical Hazard	0

NFPA Rating
Health hazard: 2

Fire Hazard:	3
Reactivity Hazard:	0

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

Preparation Information

Sigma-Aldrich Corporation
Product Safety – Americas Region
1-800-521-8956

Version: 5.4 Revision Date: 07/31/2014 Print Date: 12/01/2015

REFERENCES

(1) Hyberts, S. G.; Arthanari, H.; Wagner, G. *Topics in current chemistry* **2012**, *316*, 125-148.
(2) Arbogast, L. W.; Brinson, R. G.; Marino, J. P. *Analytical chemistry* **2015**, *87*, 3556-3561.
(3) Koumantakis, G. *Clin Biochem Rev* **2008**, *29 Suppl 1*, S61-66.
(4) Findeisen, M.; Brand, T.; Berger, S. *Magn Reson Chem* **2007**, *45*, 175-178.
(5) Strange, K. *Am J Physiol Cell Physiol* **2008**, *295*, C567-575.