

# <sup>13</sup>C Enriched Carbon Sources for Solid State NMR of Proteins

- BioExpress-1000
- Glycerol
- Glucose



## Tailoring <sup>13</sup>C Enrichment of Proteins for Solid State NMR

For many years, the use of highly enriched, uniform isotope-labeled recombinant proteins has enabled the study of protein structure and dynamics by NMR spectroscopy. This approach has been relatively easy to perform since uniformly <sup>13</sup>C labeled carbon sources such as <sup>13</sup>C<sub>6</sub> glucose have been readily available. Unfortunately, highly-enriched, uniform <sup>13</sup>C labeling of proteins is not conducive to some NMR experiments (such as solid state NMR experiments using stationary aligned samples) because strong, homonuclear <sup>13</sup>C-<sup>13</sup>C dipole-dipole coupling can act to broaden <sup>13</sup>C resonances very significantly; sometimes so severely that they are beyond detection. An effective approach to alleviate this problem is to use proteins containing spatially isolated <sup>13</sup>C sites. Such sparsely labeled proteins can either be produced using uniform, random fractionally enriched carbon sources or specific <sup>13</sup>C labeled carbon sources.

## Uniform Fractionally <sup>13</sup>C Enriched Bacterial Cell Growth Media and Glucose [U-<sup>13</sup>C<sub>6</sub>]

CIL is pleased to offer uniform fractionally <sup>13</sup>C enriched BioExpress-1000 as well as fractionally enriched glucose for those researchers that use minimal essential media instead of a ready-made rich culture medium.

| Catalog #        | Product   | Amount       |
|------------------|---|--------------|
| CGM-1000-CN-25-S | BioExpress-1000 ( <sup>13</sup> C, 25%; <sup>15</sup> N, 98%) | 10 ml (10x)  |
| CGM-1000-CN-25   | BioExpress-1000 ( <sup>13</sup> C, 25%; <sup>15</sup> N, 98%) | 100 ml (10x) |
| CGM-1000-CN-35-S | BioExpress-1000 ( <sup>13</sup> C, 35%; <sup>15</sup> N, 98%) | 10 ml (10x)  |
| CGM-1000-CN-35   | BioExpress-1000 ( <sup>13</sup> C, 35%; <sup>15</sup> N, 98%) | 100 ml (10x) |
| CGM-1000-CN-45-S | BioExpress-1000 ( <sup>13</sup> C, 45%; <sup>15</sup> N, 98%) | 10 ml (10x)  |
| CGM-1000-CN-45   | BioExpress-1000 ( <sup>13</sup> C, 45%; <sup>15</sup> N, 98%) | 100 ml (10x) |
| CLM-1396-25-1    | Glucose ( <sup>13</sup> C, 24-25%)                            | 1 g          |

The number of adjacent <sup>13</sup>C sites in most amino acid residues can be minimized by using uniform fractionally <sup>13</sup>C enriched bacterial cell growth media or glucose [U-<sup>13</sup>C<sub>6</sub>].

Opella *et al* (1) have reported optimal results using BioExpress media with fractional <sup>13</sup>C enrichments between 25% and 35%.

## Specific <sup>13</sup>C Labeled Carbon Sources

CIL offers the following specific <sup>13</sup>C labeled substrates for use in the microbial expression of proteins labeled with isolated <sup>13</sup>C sites.

| Catalog # | Compound                             | Amount |
|-----------|--------------------------------------|--------|
| CLM-1397  | Glycerol (2- <sup>13</sup> C, 99%)   | 1 g    |
| CLM-1857  | Glycerol (1,3- <sup>13</sup> C, 99%) | 1 g    |
| CLM-746   | Glucose (2- <sup>13</sup> C, 99%)    | 1 g    |

Glycerol [1,3-<sup>13</sup>C<sub>2</sub>] and glycerol[2-<sup>13</sup>C] have been shown to give rise to complementary labeling patterns. When choosing the appropriate specific <sup>13</sup>C labeled carbon source, the <sup>13</sup>C enrichment of the expressed protein can be tailored to highlight regions of interest.

## ADVANTAGES

### Glycerol [2-<sup>13</sup>C]

- increases overall labeling of carbons
- significantly lower levels of carbonyl and aliphatic side chain carbons

### Glycerol [1,3-<sup>13</sup>C<sub>2</sub>]

- creates reduced intensity in the α-carbon region (1)

### Glucose [2-<sup>13</sup>C]

- minimizes adjacent <sup>13</sup>C pairs in most residues
- reported to be more useful than specifically labeled glycerol in detecting backbone <sup>13</sup>C resonances (1)

<sup>1</sup>F.V. Filipp, N. Sinha, L. Jairam, J. Bradley, S.J. Opella Labeling strategies for <sup>13</sup>C-detected aligned-sample solid-state NMR of proteins, *J. Magn. Reson.* 201 (2009) 121-130.