Cell-Free Synthesis of Labeled Proteins for NMR Studies

Cambridge Isotopes Laboratories, Inc. (CIL) is pleased to offer kits and reagents manufactured by CellFree Sciences Co., Ltd. (CFS) for cell-free protein synthesis. The CellFree Sciences' ENDEXT® wheat germ cell-free system permits both high-throughput protein screening and overnight microgram- to milligram-scale protein production. Protein-synthesis protocols for the ENDEXT® system have been optimized for a wide range of proteins, and when used with CIL amino acids, offer an ideal platform for producing uniform or selective labeled protein for NMR applications.

Cell-free protein synthesis uses an open reaction format that allows for easy manipulation of reaction conditions to:

- add labeled amino acids for NMR studies
- add cofactors for protein(s) complexes
- add liposomes for expression of membrane proteins
- add detergents for increased solubility
- adjust redox conditions for formation of disulfide bridges

Cell-free synthesis using CFS and CIL reagents are ideal for expressing labeled:

- toxic proteins
- membrane proteins
- protein with selective incorporation of labeled amino acids
- proteins having very high incorporation rates for labeled amino acids
- proteins containing unnatural amino acids
- protein complexes composed of multiple proteins in a single reaction

Fast access to your proteins of interest starting directly from a DNA template!

Optimized expression systems for preparation of GST- and His-tagged proteins!

Easy three-step process to produce your proteins of interest:

1. Prepare expression template by PCR or cloning into expression vector
2. Cell-free protein synthesis and affinity-tag based purification
3. Protein characterization

Try cell-free protein synthesis for your next NMR sample!

How to Proceed

Step 1: Test and optimize protein expression

- Use the Premium Plus Expression Kit or a Protein Research Kit to produce unlabeled protein on a small scale.
- If desired, contact CFS for more information on cell-free protein-expression services for testing your proteins of interest.

Step 2: Express labeled proteins using cell-free protein synthesis expression kits and reagents

- Several ready-to-use WEPRO Protein Expression Kits are available for uniform ^{15}N and ^{13}C, ^{15}N labeling containing enough reagents for up to 6 x 6 mL bilayer reactions.
- WEPRO8240 Protein Expression Kits are available for producing selective or uniform labeled protein using CIL amino acids (sold separately).

Step 3: Production of bulk quantities (mg -> g) (optional)

- Cell-free protein synthesis allows rapid testing of expression conditions that can directly be used in large-scale protein production.
- Bulk program for reagent sales to large-scale user using feeding methods for protein synthesis.
- Fully automated solutions to protein synthesis on Protemist® protein synthesizers.

Contact CIL or CFS for more information on large-scale cell-free protein expression services for your proteins of interest.

Selected Publications Using CFS Products

Basics

Arumugam, T.U.; Ito, D.; Takashima, E.; Tachibana, M.; Ishino, T.; Torii, M.; Tsuboi, T. **2014**. Application of wheat germ cell-free protein expression system for novel malaria vaccine candidate discovery. *Expert Rev Vaccines, 13*(1), 75-85.

Endo,Y.; Sawasaki,T. **2006**. Cell-free expression systems for eukaryotic protein production. *Curr Opm Biotechnol, 17*(4), 373-380.

Sawasaki, T.; Ogasawara, T.; Monshita, H.; Endo, Y. **2002**. A cell-free protein synthesis system for high-throughput proteomics. *Proc Natl Acad Sci USA, 99,* 14652-14657.

Mardin, K.; Sawasaki T.; Ogasawara, T.; Endo, Y. **2000**. A highly efficient and robust cell-free protein synthesis system prepared from wheat embryos. *Proc Natl Acad Sci USA, 97,* 559-564.

Labeling

Makino, S.; Beebe, E.; Markley, J.; Fox, B. **2014**. Cell-Free Protein Synthesis for Functional and Structural Studies. *Methods Mol Biol, 1091,* 161-78.

Vinarov, D.; Loushin Newman, C.; Markley, J. **2006**. Wheat germ cell-free platform for eukaryotic protein production. *FEBS J, 273,* 4160-4169.

Vinarov, D.; Lytle, B.; Peterson, F.; Tyler, E.; Volkman, B.; Markley, J. **2004**. Cell-free protein production and labeling protocol for NMR-based structural proteomics. *Nat Methods, 1*(2), 149-153.

Membrane Proteins

Nozawa, A.; Tozawa; Y. **2014**. Modifications of wheat germ cell-free system for functional proteomics of plant membrane proteins. *Methods Mol Biol, 1072,* 259-72.

Periasamy, A.; Shadiac, N.; Amalraj, A.; Garajová, S.; Nagarajan ,Y.; Waters, S.; Mertens, H.D.; Hrmova, M. **2014**. Cell-free protein synthesis of membrane (1,3)-β-d-glucan (curdlan) synthase: co-translational insertion in liposomes and reconstitution in nanodiscs. *Biochim Biophys Acta, 1828*(2), 743-57.

Norawa, A.; Nanamlya, H.; Mlyata, T.; Linka, N.; Endo, Y.; Weber, A.P.; Tozawa, Y. **2007**. A cell-free translation and proteoliposome reconstitutlon system for functional analysis of plant solute transporters. *Plant Cell Physiol, 48,* 1815-1820.