



CellFree Sciences

Technology Application Note:

Structure Determination

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Structure Determination

ENDEXT® Technology offers several advantages over cell-based and *E. coli* cell-free protein expression systems in the structure determination of proteins.

Advantages over cell-based protein expression systems

1. ENDEXT® Technology permits protein production in excess of milligrams per milliliter of reaction volume.
2. The products can be directly used for structural studies.
3. Certain proteins incompatible with cells can be readily produced.

Advantages over E. coli cell-free protein expression system

1. Multidomain proteins, found more often in eukaryotes, stand a far better chance of folding into their correct conformations in ENDEXT® Technology than in *E. coli* system.
2. Much less contamination by degradation enzymes such as DNase, RNase, and proteases.
3. Without purification, proteins produced by ENDEXT® Technology exhibit no contamination signal in their HSQC (heteronuclear single quantum correlation) spectra, which greatly facilitates the screening of protein folding and determination of protein structures in high-throughput proteomics.

Advantages of ENDEXT® Technology over *E. coli* System in NMR Analysis

	ENDEXT®	<i>E.coli</i> (in vitro)	<i>E. coli</i> (in vivo)
Degradation by protease	no	high	high
Scrambling enzyme activity	Very low	high	high
Throughput	Excellent (BiLayer)	good	poor
Labeling efficiency	Excellent (Multi-labeling)	poor	poor
Applicability for HSQC screening	Excellent (no purification)	poor (purification required)	poor
Cost performance	Excellent	good	poor

Abbreviation

HSQC: heteronuclear single quantum correlation

ENDEXT® Technology is one of the best ways for protein structural analysis especially for NMR