E107 High-level Expression of *in vivo* Biotinylated Recombinant Proteins in Escherichia coli Using a New Series of pET-derived Expression Vectors

Jung-Hyun Park, Shin-Young Na, Eun-Wie Cho, Yun-Jung Lee, and Kil Lyong Kim
Peptide Engineering R.U., Korea Research Institute of Bioscience and Biotechnology, Yusong, P.O.Box 115, Taejon 305-600

The biotinylation of recombinant proteins and their detection with streptavidin has become an essential tool in both cell and molecular biology. In particular, the recent development of *in vivo* biotinylation techniques, where recombinant proteins are site-specifically biotinylated during their induction in *E.coli*, has opened new possibilities in the application of the biotin-avidin system. To obtain a high-efficiency expression system for the production of such *in vivo* biotinylated proteins, in the present study, a series of novel expression vectors were constructed. These vectors, termed pET-BIOTRX-II, were derived from the previously reported pBIOTRX vector, and they are designed for a T7 promoter driven expression of N-terminal single-residue biotinylated thioredoxin onto which proteins in interest can be fused using a newly introduced multiple cloning site. The fidelity of this novel system was confirmed by the recombinant expression of rat urocortin, which is a recently described neuropeptide implicated in the biological response to stress.

E108 Detection Of Apoptotic Cells Using *In Vivo* Biotinylated Annexin V

Yun-Jung Lee, Jung-Hyun Park, Shin-Young Na, Eun-Wie Cho, and Kil Lyong Kim
Peptide Engineering R.U., Korea Research Institute of Bioscience and Biotechnology, Yusong, P.O.Box 115, Taejon 305-600

Apoptosis is an active mechanism for the efficient elimination of superfluous cells. After induction of apoptosis, phosphatidyl serine residues, which were originally located to the inner surface of the cell membrane are translocated to the outer surface, and these are then specifically bound by annexin V. Using this property, sensitive assays had been developed that can detect apopototic cells by fluorescence-conjugated annexin V. In the present study, an *in vivo* biotinylated form of recombinant human annexin V is described, which enables the identification of apoptotic cells via fluorescence conjugated streptavidin and flow cytometry. For the production of such biotinylated annexin V, the cDNA encoding human annexin V was cloned by reverse transcription-PCR from whole RNA of PMA stimulated HL-60R cells and inserted into the multiple cloning site of the pET-BIOTRX II-BirA vector. This prokaryotic expression vector had been recently described by us, and enables the over-expression of *in vivo* biotinylated proteins. The fidelity of biotinylated BIOTRX-annexin V fusion proteins was confirmed with apoptosis-induced Jurkat cells, which were treated with campthothecin to induce cell death. The results from flow cytometry and confocal microscopy of these cells will be shown and discussed.