



NMR update: <sup>15</sup>N-labelled protein expression for HSQC now available!

# generon

## New Service: Protein Expression, Purification and Characterization

Generon has teamed up with a core protein expression facility within the UK. We can offer you a complete protein expression service (with full customer and trouble-shooting support) from the accession number/sequence ID all the way through to biomolecular structural analyses using any of three high efficiency expression platforms: *E. coli*, insect and mammalian (cloning, small scale expression, scale-up, final purification, tag removal, customer-directed final analyses).

From a biophysical analyses perspective, we can offer CD-spectroscopy, light scattering, mass spectrometry in addition to standard analysis tools. We now also offer a <sup>15</sup>N-labelled protein expression and purification service for <sup>15</sup>N HSQC experiment.

**Cloning Service:** Cloning (<1kb cDNA insert) from accession number- using either a cDNA library or an image clone as a template. We will design oligonucleotide primers to amplify the gene of interest and then clone this into the desired vector of choice. Constructs will be fully verified by DNA Sequencing. Prices for larger inserts available upon request. £850.00

### **Bacteria:**

**Phase 1:** A small scale pilot study which includes: bacterial transformation, expression test using both IPTG and auto-induction methods, solubility testing, SDS-PAGE gel analyses and Western blotting. £600.00

**Phase II:** 1 L scale up using the optimised conditions elucidated in Phase I to include protein expression, affinity purification, tag removal, second step purification (if required) and SDS-PAGE analyses of all protein fractions. Affinity tag will be removed if required by the appropriate enzyme digestion (eg. thrombin, TEV etc) and cleaved proteins purified by gel filtration or anion exchange chromatography. £900.00

**New <sup>15</sup>N-labelled protein expression service for protein NMR :** £550.00

An HSQC spectrum is like a nice "fingerprint" of the protein because each peak correspond to an NH of each residue. Highly purified <sup>15</sup>N labelled protein can be prepared for <sup>15</sup>N-HSQC so that you are able to determine whether the protein is folded and whether it is a good candidate for full NMR structural characterisation.

### **Insect:**

**Phase 1:** A small scale pilot study which includes: co-transfection of baculovirus DNA and transfer plasmid into Sf9 cells, P1 stock virus amplification, test infection in 6-well dishes using both Sf9 and Hi5 cell lines, at T48 and T72 hours with five concentrations of recombinant virus plus an infected control. SDS-PAGE analyses of cell fractions (total/soluble/pellet/secreted) and Western blot analysis. £900.00

**Phase II:** 0.5-1L scale up using the optimized conditions elucidated in Phase I to include: amplification to P2 and P3 of virus stocks, infection of 0.5-1L of cells, protein affinity purification, tag removal, second step purification (if required) and SDS-PAGE analyses of all protein fractions. Affinity tag will be removed if required by the appropriate enzyme digestion (eg thrombin, TEV etc) and cleaved proteins purified by gel filtration or anion exchange chromatography. £1,200.00

### **Mammalian:**

**Phase 1:** A small scale pilot study which includes: transfection into HEK293 EBNA cells and antibiotic selection of transfected cells. SDS-PAGE analyses of cell fractions (total/soluble/pellet/secreted) and Western blot analysis. £800.00

**Phase II:** 0.5-1 L scale up using the optimised conditions elucidated in Phase I to Include growth of 0.5-1L culture, affinity purification, tag removal, second step purification (if required) and SDS-PAGE analyses of all protein fractions. Affinity tag will be removed if required by the appropriate enzyme digestion (eg. thrombin, TEV etc) and cleaved proteins purified by gel filtration or anion exchange chromatography. £1,350.00

### **Biomolecular Analysis:**

Analytical characterization ensures the identity, purity, structural and conformational integrity of a protein. We perform a number of routine protein analyses throughout all project stages. We can also offer the following analyses for an additional charge: CD-spectroscopy, light scattering and mass spectrometry. £500.00

### **Overall Workflow:**

We will start by sending you a protein expression feasibility proforma asking you for specific information/ requirements e.g. nature and location of the fusion tag, preferred expression system etc. A full written purification report containing all methodologies used, together with gels and blots and certificate of analyses will be supplied together with the final purified product upon completion of the project. Where necessary, a batch record will be provided.

### **Why is Generon different to other protein expression facilities:**

- Large collection of high yielding proprietary expression vectors.
- Expression in *E. coli*, insect or mammalian expression systems.
- Standard expression (generally up to 5 mg of protein).
- <sup>15</sup>N-labelled protein expression service for HSQC-protein NMR.
- Choice of different expression tags (His, His-GST, His-NusA, His-Trx).
- High purity.
- Purified protein from 3 weeks.
- Full protein characterization using state-of-the art-instrumentation e.g. light scattering, CD spectroscopy, mass spectrometry.
- Customised service backed up by full trouble-shooting support.
- The most competitive prices in the market. We will work with you to fit within your budget.

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