ARVYS PROTEINS

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EXPERT SERVICE. TIMELY RESULTS.

Making Proteins Work for You

CONTRACT RESEARCH SERVICES

Genetic Engineering & Protein Expression
Fermentation & Cell Culture
Protein Purification
Protein Characterization
Protein Labeling & Conjugation
Endotoxin Removal & Testing

GENETIC ENGINEERING & PROTEIN EXPRESSION

High level of protein expression is critical for efficient and economical purification of active recombinant proteins. We offer a number of expression systems designed to meet specific goals, ranging from expression of tagged proteins as antigens for antibody generation to production of biopharmaceuticals for clinical use. Our experts can optimize expression by engineering an expression vector with a strong promoter, an efficient ribosome binding site, a high copy number and combining it with an appropriate host. Expression also depends on the gene sequence, stability, solubility, and folding pathway of the protein product. Accordingly, we will optimize these protein expression parameters when such optimization is necessary.

GENETIC ENGINEERING

- generation of a gene by PCR or synthesis
- engineering into an appropriate vector
- restriction mapping to confirm identity and integrity of a construct
- DNA sequencing of an insert
- plasmid preparation and DNA storage
- site-directed mutagenesis

EXPRESSION SYSTEMS

- protein expression in *E. coli*, mammalian and insect cells
- commonly used vectors and hosts for expression or systems specified by the customer
- proprietary high efficiency *E. coli* expression system offered under separate license for large scale production destined for the clinic

PROTEIN EXPRESSION OPTIONS

- stable and transient expression of secreted and intracellular proteins in mammalian cells
- secreted or intracellular proteins in insect cells
- cytosolic, periplasmic or secreted proteins in bacterial expression systems
- tagged or untagged proteins

EXPRESSION ASSESSMENT

- soluble vs. inclusion bodies analysis for bacterial cell expression systems
- SDS-PAGE, Western, ELISA, or functional analysis of cell lysates or conditioned media

EXPRESSION OPTIMIZATION

- design or selection of a vector with an efficient promoter, ribosome binding site, copy number and appropriate host
- systematic and high throughput optimization of growth conditions including media and additives selection, temperature, induction

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FERMENTATION & CELL CULTURE

Raw materials determine the purification strategy or even the success of purification. Protein extraction/recovery could be a very important purification step in itself. Time and effort invested at this stage often result in substantially shorter purification protocols and higher yields of active protein. Our experience with various methods of protein extraction, fractionation and enrichment for both recombinant and natural sources enables us to design the most optimal production process for your starting materials.

PRODUCTION OF RECOMBINANT PROTEINS

- conditioned media from insect and mammalian cell culture
- insect and mammalian cell pellet
- bacterial paste

INCLUSION BODIES REFOLDING

RAW MATERIALS ENRICHMENT

- ammonium sulfate precipitation
- sucrose gradient centrifugation
- differential detergent extraction
- partition into Triton X-114
- treatment for membrane preparations
- inclusion body isolation, wash, solubilization and refolding

CONCENTRATION OF RAW MATERIALS BY

- centrifugation
- tangential flow filtration (TFF)

QUANTITATIVE ANALYSIS OF THE TARGET PROTEIN EXPRESSION

- ELISA
- Western Blot
- Other assays upon request

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PROTEIN PURIFICATION

Our skills in protein purification were gained over years of purification of protein pharmaceuticals, drug targets, biological system components and biochemical reagents. Each protein has its own physico-chemical and biological properties that dictate a tailored extraction, purification and characterization. Our purification strategy aims at a homogeneous active protein preparation achieved in two, or less often, three purification steps by thorough selection and optimization of the capture step, incorporation of a gel filtration step to remove aggregates, degradation products and other contaminants, selection of buffer conditions that stabilize biological activity and prevent product degradation. Although most proteins require individual approach, we are confident that we can handle your protein. We will purify it cost-efficiently, characterize it according to your specifications and deliver it to you in an active and application-compatible form.

PURIFICATION FEATURES

- common purification scales range from 0.001g to 10g
- proteins are purified according to customer-specified purity
- purification methods from customer-supplied, published protocols or improved protocols
- *de novo* protocols tailored to customer's requirements
- any purification mode can be used
- purification method development for transfer to a GMP facility
- dedicated columns are used in each project
- efficiency is provided by the automation and precision of AKTA systems from Amersham BioSciences (currently GE)

ANALYSIS OF PURIFICATION

- SDS-PAGE and/or dot/Western blotting are routinely used for fraction analysis, additional assays can be requested
- final protein purity is determined by densitometry from Coomassie-stained gels
- final products are supplied with a certificate of analysis, purification report and, if applicable, a batch record

FINAL PRODUCT

- certificate of analysis is tailored to customer specification
- final products are dispensed into customer-specified aliquot sizes

AUXILIARY SERVICES

- development of affinity media
- preformulation screening
- endotoxin removal
- tag removal
- conjugation to biotin, fluorescent tag or enzyme

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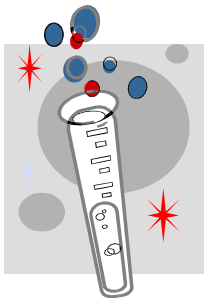
PROTEIN CHRACTERIZATION



Analytical characterization ensures the identity, purity, structural and conformational integrity, and function of the protein. We perform a number of routine protein analyses throughout all project stages. If needed, we can submit your samples for additional methods of characterization. We also offer specialized protein characterization services for purified proteins.

ANALYSIS OF RAW, INTERMEDIATE AND FINAL PROTEIN PRODUCTS

- electrophoresis (SDS-PAGE, native-PAGE, IEF-PAGE, urea-PAGE)
- Western/dot blot
- enzyme activity assays by light absorbance or fluorescence
- ELISA (direct or sandwich)
- protein assay (A_{280}, BCA or equivalent)
- antibody isotyping
- endotoxin measurement
- contaminating DNA assay
- UV-Vis absorption spectrum

PREPARATION AND SUBMISSION OF PROTEIN SAMPLES FOR

- amino acid analysis
- N-terminus analysis
- mass-spectrometry identification and analysis
- extinction coefficient determination

PROTEIN CHARACTERIZATION

- analytical size exclusion chromatography (native MW estimate, aggregation analysis)
- analytical ion-exchange chromatography (oxidation)
- purity analysis by densitometry
- UV-Vis light absorption spectroscopy
- analysis of oxidation, degradation and aggregation products
- protein deglycosylation analysis
- binding interactions by co-immunoprecipitation, spectroscopy, or chromatography

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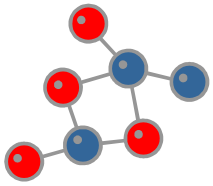
PROTEIN LABELING & CONJUGATION



Labeled proteins allow us to study specific molecular interactions with high sensitivity in complex biological systems. They are important reagents in numerous biological applications such as assays, purifications, protein arrays, localization studies, flow cytometry, clinical imaging and much more. The quality of labeled proteins is critical for consistent and reliable data. Although labeling procedures appear to be simple and straightforward, most of them still need to be adjusted to take into consideration the nature of a protein in order to achieve the desired results. Possible problems during labeling procedures include protein losses due to precipitation, sample manipulation & instability, inconsistent label-to-protein ratio, incomplete removal of an unconjugated labeling probe, and poor protein characterization before and after labeling. In addition, new labeling technologies had emerged for site-directed labeling requiring an integration of protein expression and purification into the labeling process. We have extensive experience with various protein labeling techniques and are confident that we can provide you with high-quality labeled reagents for your downstream applications.

NONSELECTIVE PROTEIN LABELING

- biotinylation with incorporation ratios determined by a HABA-based assay
- fluorescent probe conjugation with incorporation ratios determined by fluorescence
- other chemical moieties (for example, Sulfo-Tag, Dyes) with incorporation ratios determined by UV-Vis spectroscopy
- enzyme conjugation with incorporation ratios determined by enzyme activity assays

SITE-SPECIFIC LABELING

- C-terminal labeling through "Sortagging"
- N-terminal labeling through "Sortagging"
- labeling at glycosylation sites

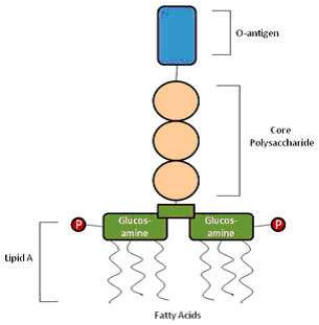
CLICK CHEMISTRY LABELING

- azido modified proteins and/or glycochains
- alkyne modified proteins and/or glycochains
- copper (I)-catalyzed click labeling
- copper-free click labeling
- various commercial choices of click partners including dual labels
- *in situ* applications
- protein conjugation and detection

BIOMOLECULE IMMOBILIZATION ON SOLID SUPPORT

- site-specific immobilization on a chip in oriented fashion
- protein conjugation to chromatography resin

ENDOTOXIN REMOVAL & TESTING



Endotoxin removal from biological solutions is critical for many *in vivo* and cell-based applications, as it interferes with biological response. Endotoxins are liposaccharides that are found in the outer cell wall of Gramm-negative bacteria. Bacteria release endotoxins at the time of lysis. The toxic effect of endotoxins is triggered by its interaction with specific receptors on the immune cells resulting in the release of high concentrations of cytokines and other molecules of immunological significance. Since adventitious endotoxin is present in air, water, labware and it cannot be removed by simple sterilization, it is almost impossible to generate endotoxin-free solutions without a removal procedure. Each endotoxin removal project requires a protocol development step that takes into consideration biophysical properties of a target molecule, final sample application and desired formulation. For years we had successfully developed endotoxin removal protocols to meet strict endotoxin presence requirements. We are confident that we can make your endotoxin removal project a success.

CUSTOM ENDOTOXIN REMOVAL FROM AQUEOUS SOLUTIONS OF

- proteins
- DNA
- peptides
- Biomass

METHODS ARE BASED ON

- charge
- hydrophobicity
- combinantion of charge and hydrophobicity
- ligand affinity
- Size

SERVICE FEATURES

- protein losses are minimized by selection of an appropriate endotoxin removal method and its optimization
- scales range from mg to g of a target molecule
- endotoxin-free samples are sterilized and distributed into multiple aliquots for convenience
- delivered in an application-compatible buffer
- turnaround times are less than 1 week in most cases
- high-throughput assay capacity
- detergent-compatible r-Factor C fluorescence-based assay
- detection levels below 0.01EU/ml
- LAL kinetic assay and other endotoxin assays are available

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WHY ARVYS?

GET ARVYS EXPERTISE

- ✓ We integrate our expertise in genetic engineering, protein expression, protein purification, protein characterization and assay development for each project to ensure project's success.
- ✓ We monitor activity and stability of proteins through every step of purification procedures thus minimizing presence of aggregated or denatured target protein in your preparations.
- ✓ Our instrumentation and experience allow us to develop a variety of biochemical and biophysical assays and use them for protein, peptide, and small molecule characterization.
- ✓ We reduce your cost by maximizing the activity and stability of your protein product.
- ✓ We deliver endotoxin-free active proteins for your *in vivo* studies.

WHY DO YOU NEED EXPERT HELP TO PURIFY AND CHARACTERIZE PROTEINS?

Your protein which appears as a single protein band on SDS-PAGE could be heterogeneous due to:

- Aggregated, modified or partially degraded forms of the target protein
- Other proteins with the same rate of migration on SDS-PAGE
- Products from faster growing cells which may come from incomplete clonal selection or clone modification
- Presence of highly active impurities which are not easily visualized, such as endotoxin

Heterogeneity in preparations results in the following problems:

- Presence of even small amounts of denatured or misfolded proteins may trigger further protein denaturation leading to instability during manipulations and storage
- Small impurities may contribute disproportionately to the product concentration measurements resulting in erroneous data interpretation
- In *in vivo* studies, denatured protein, contaminants and endotoxin may induce unwanted biological responses
- In screening experiments, denatured protein may bind inhibitors and significantly affect K_i and IC_{50} values

Your protein preparation may be fully or partially inactive due to:

- Failure to form a native conformation during production or renaturation procedures
- Denaturation, degradation or modification
- Alteration by protein tagging or labeling
- Shielding of an active site by contaminating ligands

Loss or alteration of functional activity results in the following problems:

- Ligand binding parameters are changed
- Larger amounts of protein have to be used
- Result consistency may be affected
- Increased contribution from background activity reduces assay window
- Biological responses are too low to be measured
- Your protein preparation may generate inconsistent and unreliable data.

WORKING WITH US

PROJECT SCOPE

Each project is tailored to the customer's specifications of scale, purity, analytical characterization and activity. We help our customers plan their projects in the most cost-efficient manner.

QUALITY ASSURANCE

We set high standards for our services and stay current with the latest developments in science and technology. To ensure project coordination, a project director from our senior scientific staff is assigned to every project. A project director manages all project stages – initial evaluation, quotation, planning and execution, preparation of final reports, shipments and technical support – and makes sure that a project is performed according to the customer's specifications. A project director is the customer's main contact at ARVYS.

PRICING POLICY

Our goal is to make outsourcing to ARVYS an affordable option for life scientists from both industry and academia, and yet to provide our services at the highest professional level. We are committed to being open with customers about how we price projects. Our web site has a **List of Services** where we break up our services to a single assay or a single experimental step. In our quotation we will break up our charges according to this list.

TURNAROUND TIMES

Turnaround times are provided with our official quotes. For some projects, we might break up a full project timeline into milestone timelines and provide customers with turnaround times for each step. We recognize that speed and timeliness are crucial for our clients, and we set aggressive turnaround times. At the same time, we thoroughly evaluate our human and technical resources to make sure the timelines are realistic. Once the timelines are set, we make every effort to meet them.

TECHNICAL SUPPORT

After completion of a project, we continue working with our customers for the period of 6 months to help them troubleshoot any project-related problems.

CONFIDENTIALITY

We recognize that confidentiality is vital for many of our customers. In such cases, Confidential Disclosure Agreement is executed prior to any project discussions and the following work is performed under strict confidentiality.

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