ARVYS PROTEINS

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EXPERT SERVICE. TIMELY RESULTS.

Protein Sciences Services for Critical Projects

CONTRACT RESEARCH SERVICES

- Protein Expression
- Fermentation & Cell Culture
- Protein Purification
- Protein Characterization
- Functional Assays & Assay Development
- ELISA Development
- Protein Labeling & Conjugation
- Endotoxin Removal & Testing
- Antibody Development & Production

PROTEIN EXPRESSION

Our **Protein Expression services** incorporate the latest developments in bacterial, baculovirus and mammalian expression technologies to assure that your ***recombinant protein production*** will be efficient and economical. We offer a number of **Protein ExpressionServices** designed to meet specific goals, ranging from expression of tagged proteins for research to production of biopharmaceuticals for clinical use. Your ***custom protein expression*** will be optimized by engineering an expression construct with a codon-optimized DNA insert, a strong promoter, an efficient ribosome binding site, a high copy number and combining it with an appropriate host. High level of protein expression also depends on 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
- subcloning into an appropriate vector
- confirmation of DNA insert by Sanger sequencing
- plasmid preparation and DNA storage
- site-directed mutagenesis

BACTERIAL PROTEIN EXPRESSION

- codon optimization for bacterial expression and gene synthesis of DNA insert
- mutagenesis of an existing construct
- generation of bacterial expression construct(s) in a selected vector
- transformation into an appropriate host and preparation of glycerol stocks
- screening studies for the best-expressing protein variant
- optimization of growth conditions (host, induction, media, temperature, additives) to drive either soluble or inclusion bodies expression
- soluble vs. inclusion bodies expression assessment by SDS-PAGE/Coomassie or Western blot
- bacterial paste scale up production
- inclusion bodies wash and recovery
- recombinant protein purification from soluble lysate fraction
- recombinant protein purification from inclusion bodies by refolding

BACULOVIRUS-INDUCED INSECT PROTEIN EXPRESSION

- codon optimization for insect cell expression and gene synthesis of a DNA insert mutagenesis of an existing construct
- subcloning of a DNA insert into a selected baculovirus expression vector
- virus generation, amplification and cloning by limited dilution or plaque purification
- generation of high-titer virus stocks
- SDS-PAGE, Western, ELISA or functional expression analysis of cell lysates and conditioned media for intracellular and secreted protein products respectively
- secreted or intracellular expression in *sf9*, *sf21* and *HiFive* insect cells
- expression optimization studies: MOI, time course, growth media
- large-scale insect cell culture for conditioned media or cell pellet production
- recombinant protein purification from conditioned media or cell pellet

continued

PROTEIN EXPRESSION

continued

YEAST PROTEIN EXPRESSION

- codon optimization for yeast cell expression and gene synthesis of a DNA insert
- subcloning of a DNA insert into a selected yeast expression vector
- mutagenesis of an existing construct
- generation of yeast transformants
- screening for high-yielding transformants by Western, ELISA or functional expression analysis of cell lysates
- intracellular expression of membrane proteins in *S.cerevisiae* yeast cells
- secreted expression in *Pichia* yeast cells
- expression optimization studies: secretion signal, host strain, media
- large-scale yeast cell culture for conditioned media or cell pellet production
- recombinant protein purification from conditioned media or cell pellet

MAMMALIAN PROTEIN EXPRESSION

- codon optimization for mammalian cell expression and gene synthesis of a DNA insert
- mutagenesis of an existing construct
- generation of mammalian protein expression construct(s) in a selected vector
- endotoxin-free plasmid DNA preparation for transient or stable transfections
- small-scale trial to assess recombinant protein expression and/or to optimize transient transfection conditions
- various transient transfection reagent options
- generation of stably-transfected cell pool
- expression analysis by SDS-PAGE, Western, ELISA or functional assay
- secreted or intracellular expression in CHO, HEK293 or client-specified cells
- adherent or suspension cell growth options
- large-scale mammalian cell culture for conditioned media or cell pellet production
- recombinant protein purification from conditioned media or cell pellet

STABLE CELL LINE DEVELOPMENT

AUXILLARY PROTEIN EXPRESSION SERVICES INCLUDE

- long-term storage for DNA, glycerol and virus stocks
- master and working cell banking

FERMENTATION & CELL CULTURE

The quality of starting materials resulting from **Fermentation Scale Up** often determine the purification strategy or even the success of purification. Our **Cell Culture Services** aim at achieving maximal r-protein production through optimization of fermentation processes and it 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 r-protein. Our experience with various high-producing cell lines, cell culture growth modes, media formulations, methods of protein extraction, fractionation and enrichment enables us to design the most optimal production process for your starting materials.

LARGE SCALE PLASMID DNA PREPARATION

LARGE SCALE PROTEIN PRODUCTION

- clarified conditioned media from mammalian, yeast and insect cells
- mammalian cell pellet
- insect cell paste
- bacterial paste
- yeast cell paste
- transient transfection into mammalian cells
- infection of insect cells with high-titer baculovirus
- propagation of stably-expressing insect or mammalian cells
- suspension cell line growth in shake flasks and Wave bioreactors
- adherent cell line growth in multifloor flasks and cell factories

CELL LYSIS OPTIONS

- sonication
- homogenization
- detergent solubilization
- hypotonic solution treatment
- combination of the above

INCLUSION BODIES REFOLDING SCREEN

TARGET PROTEIN ENRICHMENT BY

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

CONCENTRATION AND BUFFER EXCHANGE OF CONDITIONED MEDIA BY

- centrifugation
- tangential flow filtration (TFF)

QUANTITATIVE ANALYSIS OF THE TARGET PROTEIN EXPRESSION

PROTEIN PURIFICATION

Our skills in ***custom protein purification*** were gained over many years of work with protein pharmaceuticals, drug targets, biological system components and biochemical reagents. All **Protein Purification Services** start with the analysis of physico-chemical and biological properties of a target protein resulting in the development of tailored procedures for its extraction, purification and characterization. Our purification strategy aims to achieve a homogeneous active protein preparation in two to three purification steps. This goal is reached by a 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. We routinely work with antibodies, antigens, enzymes, growth factors, DNA-binding proteins, membrane proteins, blood proteins and many more. Some of Although most proteins require an 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.

CUSTOM PROTEIN PURIFICATION FEATURES

- purification of
  - fusion and tagged proteins from recombinant sources
  - native proteins from natural sources
  - antibodies of different isotypes including IgMs
  - membrane proteins
- common protein purification scales range from 0.001g to 5g
- proteins are purified according to client-specified purity
- protein purification methods from
  - client-supplied protocols
  - published protocols
  - improved protocols from client-supplied or published protocols
  - *de novo* protocols tailored to client's requirements
- any protein purification mode can be used
  - ion-exchange
  - gel filtration
  - affinity (broad-spectrum)
  - hydrophobic interaction
- refolding from inclusion bodies
- protein 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 INTERMEDIATE AND FINAL PURIFIED PROTEINS

SDS-PAGE and/or dot/Western blotting are routinely used for fraction analysis
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

continued

PROTEIN PURIFICATION

continued

ALL CUSTOM PURIFIED PROTEINS ARE

- supplied with a certificate of analysis tailored to client's specification
- provided at specified protein concentration
- formulated in a buffer that protects from protein degradation due to proteolysis, oxidation and shear stress
- dispensed into specified aliquot sizes

AUXILIARY PROTEIN PURIFICATION SERVICES INCLUDE

- protein refolding
- endotoxin removal
- tag removal
- labeling & conjugation

PROTEIN CHARACTERIZATION

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 F/NAL 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

FUNCTIONAL ASSAYS & ASSAY DEVELOPMENT

We offer our experience in **Functional Assays & Assay Development** for detailed mechanistic studies of a target protein, bioanalytical measurments and high-throughput screening. Since we thoroughly characterize the materials for which an assay is developed, our assays have maximized signal windows, low variability and are economical. These assays can have diverse applications such as analysis of enzyme activities, small molecule binding to proteins, protein-protein interactions and nucleic acid-protein interactions. In addition, we offer our services for outsourcing your assays or mechanistic studies.

FUNCTIONAL MODES

- protein-protein, protein-nucleic acid, protein-small molecule or peptide binding interactions
- receptor functional assays
- enzymatic activity
- direct, indirect, sandwich, and competitive ELISA
- inhibition

ASSAY FORMATS

- 96-well plate
- single sample (for UV/Vis absorbance)
- HTS-compatible

PREFERRED DETECTION METHODS

- fluorescence intensity
- fluorescence polarization
- fluorescence energy resonance transfer (FRET)
- luminescence
- light absorbance

ASSAY TARGETS

- enzymes
- antibodies
- receptors
- inhibitors
- peptides
- small molecules

ASSAY DELIVERABLES

- binding site characterization (B_{max} and J_d)
- enzyme steady state kinetic parameters determination (K_m and V_{max})
- inhibitor characterization
- single point inhibition for multiple inhibitors at a specified concentration
- IC_{50} and K_i determination
- analysis of tight binding inhibitor kinetics
- concentration determination for proteins, peptides and small molecules

ELISA DEVELOPMENT

Enzyme-Linked Immunosorbent Assay (**ELISA**) is a widely used assay in diagnostics, drug discovery, clinical development and other life-science industries. This is a sensitive assay that detects and quantifies a target molecule in biological fluids or solutions. **ELISA** can be performed in various formats and platforms. Although a target-specific antibody is the central aspect of an **ELISA**, other components are also critical for the assay performance. Our seamless, integrated expertise in antibody-antigen biochemistry, functional assays, recombinant protein production, hybridoma cell culture, antibody purification, characterization and conjugation allows us to deliver a fully optimized and validated **ELISA** for its intended final application.

ELISA TYPES

- direct
- indirect
- sandwich
- competition
- inhibition

ELISA DETECTION OPTIONS

- visible absorbance
- fluorescence
- luminescence

ELISA DESIGN INCLUDES SELECTION OF:

- assay format
- antibody or antibody pair
- standards
- plate-coating chemistry
- blocking and wash buffers
- detection strategy
- detection reagents

ELISA OPTIMIZATION INVOLVES TESTING OF VARIOUS:

- concentrations of antibodies, samples and buffers
- coating protocols
- detection protocols
- chessboard titrations

ELISA VALIDATION PARAMETERS ARE TESTED:

- robustness / precision / trueness
- uncertainty
- limit of quantification
- dilutional linearity
- parallelism
- recovery
- selectivity
- sample stability

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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
- 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
- detergent-compatible r-Factor C fluorescence-based assay
- detection levels below 0.01EU/ml
- LAL kinetic assay and other endotoxin assays are available

ANTIBODY DEVELOPMENT & PRODUCTION

In the last 30 years, **Antibodies** had become very important reagents in research, diagnostics, and medicine. This class of molecules are heavily used now in many fields of biomedical research such as immunoassays (Western blots, ELISA, etc.), protein purification, protein identification and protein-protein interaction studies. Many commercial diagnostic kits have ***antibodies*** as their critical components. Currently, there are 75 **Monoclonal Antibodies** or their derivatives approved by the FDA and many more are being tested in clinical studies. Each ***antibody*** project requires a customized approach gathering all facts about an antigen and defining the properties of the final ***antibody***. We have a long history in developing and producing ***antibodies*** for our clients. We will plan your **Antibody** project thoroughly to achieve desired outcomes.

PURIFICATION OF ANTIBODIES FROM BIOLOGICAL SOURCES

- all antibody isotypes from any species
- endotoxin removal from antibody solutions
- antibody conjugation to fluorescent dyes or enzymes
- antibody characterization by ELISA and other assays

PRODUCTION OF RECOMBINANT ANTIBODIES IN MAMMALIAN EXPRESSION SYSTEMS BY TRANSIENT OR STABLE TRANSFECTION

PRODUCTION OF MONOCLONAL ANTIBODIES FROM HYBRIDOMA CELL LINES

PRODUCTION OF POLYCLONAL ANTIBODIES

- design of an antigen with immunogenic properties
- antigen generation, production and purification
- analysis of bleeds for specificity by ELISA and/or Western blot
- purification of antibodies from blood plasma

PRODUCTION OF SINGLE CHAIN ANTIBODIES (scFv) and Fab FRAGMENTS IN *E.COLI*

- design of an expression construct
- expression in *E.coli* and its optimization
- antibody purification

continued

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ANTIBODY DEVELOPMENT & PRODUCTION

continued

GENERATION OF Fab AND (Fab)2 FRAGMENTS FROM FULL LENGTH ANTIBODIES

- optimization of enzymatic digestion reaction
- removal of heavy chain fragments
- Fab and (Fab)2 purification

PHAGE DISPLAY FOR GENERATION OF MONOCLONAL ANTIBODIES

- generation of antibody phage display library from infected/diseased patients or antigen-immunized animals
- phage display panning and screening
- sequence analysis
- generation of scFv, Fabs or whole immunoglobulins in various expression systems

CHARACTERIZATION OF ANTIBODIES FOR SPECIFICITY AND SELECTIVITY

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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

OUR BUSINESS MODEL IS SIMPLE:

- ✓ Scientific and technical excellence
- ✓ Financial stability by being self-sufficient
- ✓ Do not get involved in mergers, acquisitions ; stay away from infusion of external capital
- ✓ Build operations with only one goal in mind – servicing our clients
- ✓ Integrity and ethics are our core values

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 quotes break up our charges by milestone s. Each milestone is a "Yes / No" decision point for a client.

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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