This offer is valid for the development of stable CHO cell pools for the production of recombinant proteins via stable gene expression (SGE) in CHO cells

Included Services:	- cDNA-synthesis and cloning into expression vector - Generation of three stable CHO cell pools via a two-step selection process - Fed-batch production in serum-free suspension culture - One-step purification by affinity chromatography - Quality control by gel electrophoresis (CGE or PAGE) + photometric ($A_{280\ nm}$) or calorimetric (BCA assay) determination of protein concentration
Deliverables:	- Up to 1 mg purified recombinant protein per selected cell pool - Certificate of Analysis
Turnaround Time:	14-18 weeks
Price:	**On Request**

INSTRUCTIONS

Please complete this form and send it to info.invivo@bruker.com. Fields marked with an asterisk are mandatory. Not available or confidential information can be marked with "n/a".

CONTACT INFORMATION

	Billing Address	**Delivery Address** *(if different)*
Name*		
Company or Institution*		
Department		
Address*		
Phone*		
Email*		
VAT Number*		

PROTEIN AND SEQUENCE INFORMATION

Name*	
Accession Number*	
Species of Origin*	☐ Mouse ☐ Rat ☐ Rabbit ☐ Human ☐ Other: ***Please note:*** *InVivo only handles genetic material which originated from S1- level organisms*
Protein Location*	☐ Secreted ☐ Cytoplasmic ☐ Membrane-bound
Amino Acid Sequence*	
cDNA Synthesis*	☐ No special requirements ☐ Express synthesis (*extra costs apply*)

Please specify below, if any protein features may cause difficulties in either protein expression or purification.

DNA DESIGN, CLONING AND PLASMID PREPARATION

For efficient SGE in CHO cells, InVivo uses a proprietary expression vector; cDNA synthesis is performed by a subcontractor. The DNA-sequence is codon optimized for expression in CHO cells; restriction sites for subsequent cloning are added, as well as signal peptides for efficient protein secretion.
The preparation of transfection-grade plasmid DNA occurs via InVivo´s own plasmid preparation method.
For protein purification via affinity chromatography an additional tag can be chosen (Refer to "Protein Purification" section). If requested, this tag can be removed after protein purification via protease cleavage. In this case additional costs for protease cleavage and a secondary purification step will apply.

CULTIVATION, TRANSFECTION AND PRODUCTION

For cultivation of CHO cells, a chemically-defined, animal component- and serum-free media is used. Transfection occurs via transposon-mediated active gene transfer. CHO suspension cells are co-transfected with the expression vector encoding the protein (pPB-mono; piggy-Bac donor plasmid) and the piggy-Bac helper plasmid encoding the transposase. Through a two-step selection process, three stable CHO cell pools are generated. The customer obtains samples from all three pools (cell culture supernatant or purified material) for evaluation and selects the best pool for further productions. Subsequently, from the selected pool a 1 L fed-batch production is performed using InVivo standard protocols.

PROTEIN PURIFICATION

One-step protein purification is performed according to InVivo standard protocols. The final product is sterile filtered (standard concentration ≥ 0.5 mg / mL) and stored in PBS buffer, pH 7.4 w/o additives.

Endotoxin-free purification can be performed if needed.

Tag*	☐ His ☐ GST ☐ Fc ☐ Other:
	☐ Tag-removal via protease cleavage ☐ Tag-removal not necessary
Endotoxin Limit*	☐ Determination of endotoxin-level ☐ Endotoxin-free purification (aim: < 10 EU/mg)

Have you already established a specific protocol for protein purification? Please specify below.

If other special requirements apply for protein purification (e.g. IEX, HIC, Reverse Phase Chromatography, etc.) or dialysis, then please provide this information below. Note that additional costs may apply.

QUALITY CONTROL

For quality control, protein concentration is determined via photometric measurement ($A_{280\ nm}$) or calorimetric (BCA assay) measurement. Purity is analysed via CGE (aim ≥ 90%) or shown as SDS-Page. Storage and delivery occur in bulk at 2-8 °C.

If special services are needed for quality control (e.g. determination of purity via analytical SEC) or specific requirements apply for quality (e.g. a defined purity level and/or concentration), storage conditions (e.g. storage at ≤ -15°C, a defined final buffer and/or aliquot sizes) or shipment, then please provide this information below. Note that additional costs may apply.

LABEL (EXEMPLARY)

ADDITIONAL COMMENTS

EXPORT CONTROL STATEMENT*

With reference to Council Regulation (EU) No 2021/821 of the EUROPEAN PARLIAMENT and of the council of 20 May 2021 setting up a Community regime for the control of exports, transfer, brokering and transit of dual-use items, your action is required.

Please indicate whether the item to which this form relates (e.g. antigen, or antibody, or derivatives or progenies thereof) is suitable for the detection of biological agents (e.g. pathogens or toxins) listed in Category 1 Class C of Annex I to Council Regulation (EU) No 2021/821 under positions 1C351, 1C353 or 1C354 (see link: Publications Office (europa.eu).

☐ *Suitable*
☐ *Not Suitable*

Name (and Title)*	
Affix Company Stamp*	
Place and Date*	
Signature*	

In case the item is suitable for the detection of biological agents listed in Annex I to Council Regulation (EU) No 2021/821, then please provide further information regarding the product name, product information and export list number:

Product Name	
Product Information	
Export List Number	