Order Processing Form

Eigner: Customer Service

Rec P Pilot Production in *E. coli*

This offer is valid for one small-scale test production of recombinant proteins in *E. coli*

Included Services:
- cDNA-design and cloning into expression vector
- Gene expression in *E. coli*
- Multi-step purification
- Quality control by gel electrophoresis (CGE or PAGE) + photometric ($A_{280\ nm}$) or calorimetric (BCA assay) determination of protein concentration

Deliverables:
- Up to 20 mg purified protein (no yield guarantee)
- Certificate of Analysis
- *Optional:* synthesized cDNA in cloning vector (1-2 µg)

Turnaround Time: > 8 weeks[1]

Price: **€ 3 495.00** plus costs for cDNA synthesis[1]

For special or additional services extra costs apply

[1]*Turnaround time and costs for cDNA synthesis depend on the protein sequence length, and are provided after evaluation of submitted data*

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	☐ Unknown ☐ Inclusion bodies ☐ Soluble
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 AND CLONING

For efficient gene expression, InVivo uses a proprietary expression vector; cDNA synthesis is performed by a subcontractor. The DNA-sequence is codon optimized for expression in *E. coli* and restriction sites for subsequent cloning are added.

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

Cultivation of transgenic *E. coli* strains occurs in 2.4 L rich media in shake flasks according to InVivo standard protocols.

If specific growth conditions (e.g. additives, a defined growth temperature or specific media) are required, then please provide this information below and specify the concentration if applicable.

PROTEIN PURIFICATION

Protein purification is performed via affinity chromatography according to InVivo standard protocols, potentially followed by preparative SEC. The final product is sterile filtered and stored in PBS buffer, pH 7.4 w/o additives (standard concentration ≥ 0.5 mg/mL).

Tag*	☐ His ☐ GST ☐ Fc ☐ Other:
	☐ Tag-removal via protease cleavage ☐ Tag-removal not necessary

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

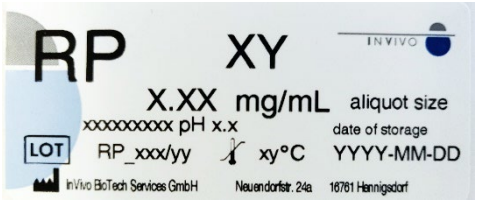
If other special requirements apply for protein purification (e.g. IEX, HIC, Reverse Phase Chromatography, etc.) or dialysis, then please provide this information below.

QUALITY CONTROL

For quality control, protein concentration is determined via photometric ($A_{280\ nm}$) or calorimetric (BCA assay) measurement. Purity is analysed via CGE (aim ≥ 90%) or shown via SDS-PAGE. Storage and delivery occurs 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 (EC) No 428/2009 of 5 May 2009 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 (EC) No 428/2009 under positions 1C351, 1C353 or 1C354 (Link).

☐ *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 (EC) No 428/2009, then please provide further information regarding the product name, product information and export list number:

Product Name	
Product Information	
Export List Number	