

Order Processing Form

Eigner: Customer Service

Rec P Pilot Production in HEK Cells

This offer is valid for one small-scale test production of recombinant proteins via transient gene expression (TGE) in HEK cells

Included Services:	<ul style="list-style-type: none"> - cDNA-design and cloning into expression vector - Transient gene expression in HEK cells - Multi-step purification - Quality control by gel electrophoresis (CGE or PAGE) + photometric ($A_{280\text{ nm}}$) or calorimetric (BCA assay) determination of protein concentration
Deliverables:	<ul style="list-style-type: none"> - Up to 20 mg purified protein (no yield guarantee) - Certificate of Analysis - <i>Optional:</i> synthesized cDNA in cloning vector (1-2 μg)
Turnaround Time:	> 8 weeks ¹
Price:	<p>€ 3 995.00 plus costs for cDNA synthesis¹</p> <p><i>For special or additional services extra costs apply</i></p>

¹Turnaround 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*		

Gültigkeitsdatum: 23.03.2020
 Änderungskontrollnummer: DCR-00992

Autor/Datum: Dr. Susanne Wolfenstetter / 18.03.2020
 Freigabe/Datum: Janina Vincenz / 19.03.2020

PROTEIN AND SEQUENCE INFORMATION

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

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

DNA DESIGN, CLONING AND PLASMID PREPARATION

For efficient TGE in HEK cells, InVivo uses a proprietary expression vector; cDNA synthesis is performed by a subcontractor. The DNA-sequence is codon optimized for expression in mammalian 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 AND TRANSFECTION

Cultivation of HEK cells occurs in suspension in 1 L shake flasks under serum-free conditions according to InVivo standard protocols. Transfection of cells is performed using **INVect** transfection reagent. The expression culture is subsequently propagated with an appropriate feeding strategy.

If specific growth conditions (e.g. vitamins or other additives) are required, then please provide this information below and specify the concentration.

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).

Endotoxin-free purification can be performed if needed, but extra charges apply.

Tag*	His GST Fc Other:
	Tag-removal via protease cleavage Tag-removal not necessary
Endotoxin Limit*	No special requirements Endotoxin-free purification (< 10 EU/mg)

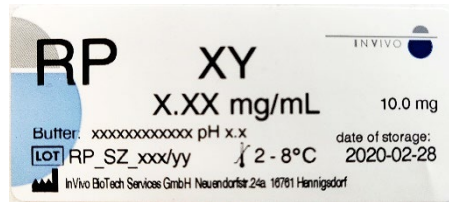
Have you already established a specific protocol for protein purification? Please specify below.

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 ($A_{280\text{ nm}}$) or calorimetric (BCA assay) measurement. Purity is analyzed via CGE (aim $\geq 90\%$) or shown as 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 $\leq -15^\circ\text{C}$, 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**

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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	