

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

<b>Included Services:</b>	<ul style="list-style-type: none"><li>- cDNA-synthesis and cloning into expression vector</li><li>- Generation of three stable CHO cell pools via a two-step selection process</li><li>- Fed-batch production in serum-free suspension culture</li><li>- One-step purification by affinity chromatography</li><li>- Quality control by capillary gel electrophoresis (CGE) + analytical size exclusion chromatography (SEC) + photometric (<math>A_{280\text{ nm}}</math>) determination of protein concentration</li></ul>
<b>Deliverables:</b>	<ul style="list-style-type: none"><li>- 10 mg purified recombinant antibody per selected cell pool</li><li>- Certificate of Analysis</li></ul>
<b>Turnaround Time:</b>	12-16 weeks
<b>Price:</b>	<b>On Request</b> <i>For special or additional services extra costs apply</i>

#### INSTRUCTIONS

Please complete this form and send it to [info.invivo@bruker.com](mailto: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*		

## ANTIBODY AND SEQUENCE INFORMATION

<b>Name*</b>	
<b>Species of Origin*</b>	Mouse    Rat    Rabbit    Human    Other:
<b>Amino Acid Sequence LC*</b>	
<b>Amino Acid Sequence HC*</b>	
<b>cDNA Synthesis*</b>	No special requirements  Express synthesis ( <i>extra costs apply</i> )

Optional service: sequencing of antibodies from existing hybridoma cell line to obtain sequence information (full-length) for subsequent transfer into a recombinant antibody. Please get in touch with the customer service team for more detailed information (Lead time: approx. 4-6 weeks).

## 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.

## 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 both recombinant antibody chains (pPB-double; 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 and antibody titers are measured from the cell culture supernatant by analytical affinity chromatography via Protein A. The customer obtains samples (cell culture supernatant or purified material) from all three pools 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 via antibody-specific affinity chromatography according to InVivo standard protocols. Elution occurs via citric acid (pH adjustment via  $K_3PO_4$ ).

The final product is sterile filtered (standard concentration  $\geq 0.5$  mg / mL) and stored in PBS buffer, pH 7.4 w/o additives.

For polishing, multi-step protein purification (IEX or preparative SEC) can be performed if needed, but additional costs apply. Also, endotoxin-free purification can be performed at additional costs.

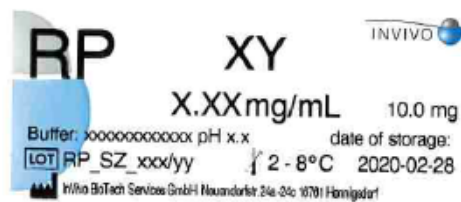
<b>Polishing*</b>	Not required  Multi-step purification; please specify:
<b>Endotoxin Limit*</b>	No special requirements  Endotoxin-free purification (< 10 EU/mg)

### QUALITY CONTROL

For quality control, protein concentration is determined via photometric measurement ( $A_{280\text{ nm}}$ ), purity is analysed via CGE (aim  $\geq 90\%$ ) and aggregation level via analytical SEC. Storage and delivery occurs in bulk at 2–8 °C.

If special 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

**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 \(europa.eu\)](https://publications.office.europa.eu)).

Suitable

Not Suitable

<b>Name (and Title)*</b>	
<b>Affix Company Stamp*</b>	
<b>Place and Date*</b>	
<b>Signature*</b>	

**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:**

<b>Product Name</b>	
<b>Product Information</b>	
<b>Export List Number</b>	