Expression of Difficult-to-Express (DTE) Proteins

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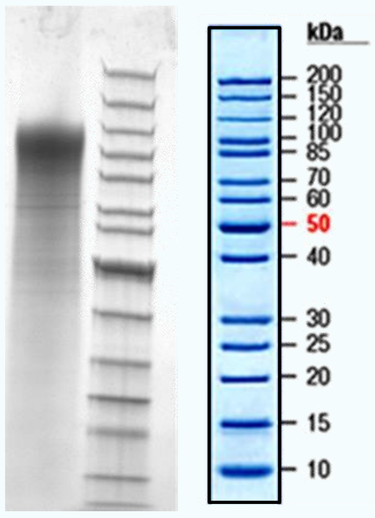
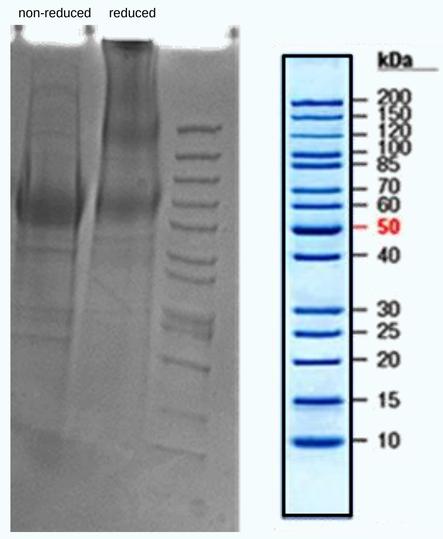
Virus Proteins

One of the greatest medical interventions of the last century is vaccination. Today vaccines are available for nearly the half of more than seventy known human infectious diseases. A vaccine is an antigen which typically mimics the structure of a pathogen. The immune system recognizes, attacks and memorizes the pathogen. Different types of vaccines are available. Live attenuated vaccines are very efficient but potential risky, inactivated vaccines are minor risky but immune response could be lost and vaccination by purified subunits is very safe but needs high amounts of antigen for several doses. Accordingly, there is a strong desire for further improvements in vaccination. Nowadays recombinant vaccines are within the focus of development. Recombinant vaccines range from recombinant subunit vaccines, virus-like particles and viral vectors to DNA vaccines. Using InVEST, InVivo has the ability to produce difficult-to-express virus proteins with enhanced glycosylation pattern in high yields for basic research to accelerate vaccine development.

Human respiratory syncytial virus attachment protein (26 kDa)
27 glycosylation sites



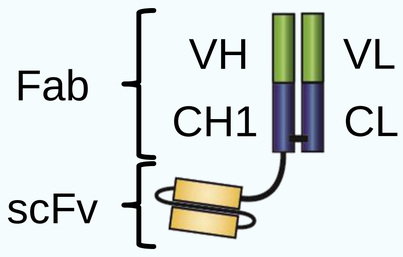
Human immunodeficiency virus 1
gp120 (54 kDa)
25 N-linked glycosylation sites



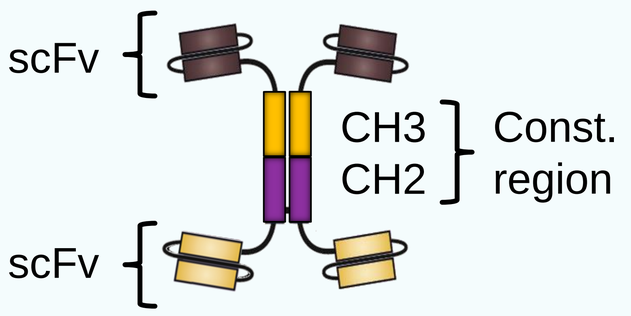
Novel Antibody Formats

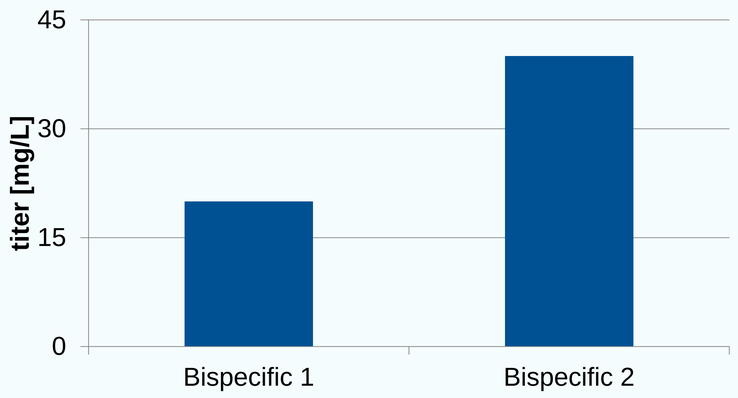
Trends in antibody discovery indicate that there is a shift to novel antibody formats in diagnostic and clinical applications. The architecture of those formats range from 12 – 150 kDa with up to tetrameric (or even higher) valency and different specifity. Most novel antibody formats are based on the single-chain variable (V)-domain antibody fragment (scFv) which comprises V domains from the heavy and light chain (VH and VL domain) joined by a peptide linker. However, some formats like bispecific scFv-Fc antibodies forming dimers with tetrameric valency which are hard to express in a functional way. Currently, more than 30 bispecific antibodies with various designs are within clinical development pipeline and also multispecific antibodies show promising results. Using InVEST InVivo has the ability to contribute to the emerging field of novel therapeutic concepts regarding multispecific antibody formats and to overcome main production issues of these formats such as correct folding, aggregation and other impurities.

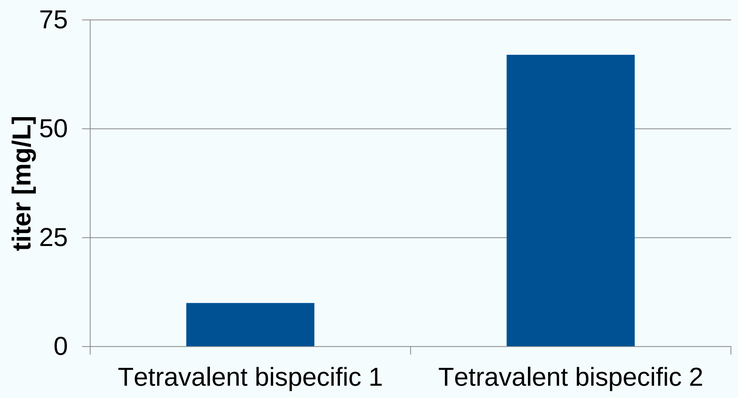
Bispecific



Tetravalent and Bispecific



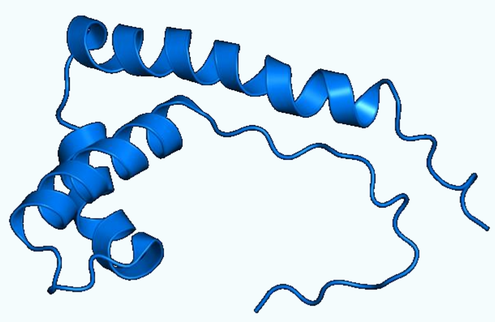




Cytotoxic Proteins

Cytotoxic proteins such as cytokines or proteases show increasing interest in basic research, diagnostic and other applications. While stable cell line development of these proteins is still challenging, transient gene expression is the method of choice.
On the one hand the high mobility group box 1 (HMBG1) protein belongs to the chromatin proteins and interacts with nucleosomes, transcription factors and histones and on the other hand secreted HMBG1 acts as a chemokine. Expression of HMBG1 is proven to be difficult in a functional and sufficient way in mammalian cell systems.
Due to its natural behavior, expression of proteases in a functional way is difficult. Proteases are enzymes for proteolysis and occur in all organisms. Since these proteins determine the lifetime of other proteins, their native expression is highly regulated and therefore it is clear that overexpression might lead to a significant reduction in viability during production phase.
However, using our process optimized transient production platform InVEST we are able to generate significant amounts of functional cytotoxic proteins in mg/L scale.

HMBG1



Reference: Protein Data Bank in Europe (https://www.ebi.ac.uk/pdbe/entry/pdb/2rtu/analysis)

Protease