



Recombinant antibody platform

1: Input;

Sequences of heavy and light chain.

Obtained from:

Hybridoma

Phage display

Other techniques

2: Identification;

Sequences of variable domains.

3: Gene synthesis;

Codon optimized for expression host.

Amino acid substitutions.

4: Initial recombinant antibody production batch;

*Transfection to: * HEK293E*

** CHO-potelligent*

Harvest.

ProteinA purification.

Gelfiltration polishing step.

5: Expression optimization:

To maximize production yield per liter.

6: Large scale production batch:

Transfection to HEK293 or CHO (max 60 liter).

Harvest.

ProteinA purification.

Gelfiltration polishing step.

Analysis and formulation.

At U-Protein Express BV recombinant antibody production starts with the identification of the variable domains of the heavy and the light chain. Synthetic, codon optimized genes for the variable domains of the heavy and light chain are synthesized and cloned into antibody expression vectors.

Next, expression vectors for the heavy and light chain are transiently co-transfected to HEK293E cells or CHO-potelligent cells from BioWa. CHO-potelligent cells produce recombinant antibodies that are 100% fucose-free, thereby enhancing Antibody Dependent Cellular Cytotoxicity (ADCC), a critical factor in anti-tumor activity. Transfection can be done at a small scale (<100 ml) to determine optimal expression conditions or directly at a large scale (1 Liter to 60 Liter).

One week post transfection conditioned medium is harvested and the recombinant antibody is purified by proteinA affinity and gel filtration chromatography.

Finally, the purity of the recombinant antibody is estimated by NuPage electrophoresis and endotoxin levels can be determined when required. Functionality of the recombinant antibody can be studied by ELISA or biointeraction analysis using an Octet biosensor.

Advantages:

* Fast, only 4 weeks from cloning of the variable domain sequences to purified recombinant antibody (design and synthesis of codon optimized variable genes adds an additional 2-3 weeks).

* Flexible, heavy and light chain variable domains can be cloned into expression vectors for any immunoglobulin subclass.

* High throughput.

* High yield, average expression level in HEK293E is 60 mg/L, highest expression level obtained is 170 mg/L.

* Yield in CHO-potelligent varies between 0.5 and 2.0 mg/L, enabling rapid production for ADCC tests and target identification prior to cell line generation.

* Experience with over 200 recombinant antibodies.

Contact information

U-Protein Express B.V.

H.R. Kruijtbouw

Padualaan 8

3584 CH Utrecht

The Netherlands

+31-(0)30-253-7903

info@u-proteinexpress.com

www.u-proteinexpress.com