STABLE CELL LINE DEVELOPMENT

STABLE CELL POOL DEVELOPMENT

1

DNA-design and cloning

Based on customer reference sequence, the target DNA is designed, optimized and synthesized, followed by cloning into InVivo's proprietary expression vector. DNA preparation occurs using a sustainable and optimized in-house process to win excellent transfection grade plasmid DNA.

3-4 weeks

2

PEI-mediated transfection

The expression vector is introduced into CHO cells via PEI-mediated transfection. Through transposon-mediated active gene transfer, the target DNA is stably inserted in the host cell genome.

~ 1 week

3

Selection of stable expressors

To select for pools with stable expression of the target DNA, double selection with puromycin and MTX is performed. The pools with best productivity are chosen and secured.

4-5 weeks

4

Selection of best pool

Small-scale test productions with the generated cell pools are performed. Productivity is determined by analytical chromatography, and the best producing cell pool is identified.

2-3 weeks

5

Single Cell Cloning

The selected cell pool is used for limited dilution to isolate monoclones. Clonality screening occurs with the NyONE FL10 imager; productivity is measured by analytical biolayer interferometry (Octet) and chromatography.
Up to 3 monoclones are selected and expanded.

12 weeks

6

Clone stability testing

The selected clone is cultivated over 60-70 population doublings and the clone expression stability is evaluated by analytical chromatography.

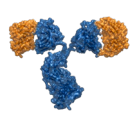
10-12 weeks

7

Upscaling production

The best monoclone is used for upscaling productions dependent on customer requirements (fed-batch shaker assay or fed-batch fermentation in bioreactor).

5-8 weeks



5

Upscaling production

The best producing cell pool is used for upscaling productions dependent on customer requirements (fed-batch shaker assay or fed-batch fermentation in bioreactor).

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