

Cell line development Fact Sheet

Viropro offers cell-line and process development services using various expression systems and cell lines designed for biopharmaceutical manufacturing.

Cell Line and Expression System Characteristics:

Cumate gene switch:

- Inducible expression using cumic acid, a small water soluble, non-toxic, molecule that is removed with the first DSP step; concentration = 1 mg/liter for induction;
- High expression, low copy number expression system (avg. of 1-2 copies/clone);
- The CR5 promoter is more efficient at 30° C and generates a 2x yield increase;
- Final MAb production levels are 5x higher than levels obtained with an enhanced CMV promoter (variations are greater still for smaller proteins);
- The cell line is derived from CHO-S, grows well in a diversity of serum-free and CD media in static and bioreactor conditions.

Other expression systems:

- Novel GS based system,
 - The cell line is derived from CHO-DG44 and is adapted to industrial requirements;
- Constitutive low copy number expression system based on CMV5 promoter;

Task Name	Duration	Start	Finish
1 A. Gene optimization (Private company)	20 days	Mon 1/4/10	Fri 1/29/10
2 B. Clonal selection	185 days	Mon 2/1/10	Fri 10/15/10
3 B1. Initial cloning & sequencing	19 days	Mon 2/1/10	Thu 2/25/10
4 Cloning of two sequences	14 days	Mon 2/1/10	Thu 2/18/10
5 Sequencing (1 heavy + 1 light chains)	5 days	Fri 2/19/10	Thu 2/25/10
6 B2a. Transient expression study (rcTA)	15 days	Fri 2/26/10	Thu 3/18/10
7 PI Transfection (293 HEK cells) + growth	11 days	Fri 2/26/10	Fri 3/12/10
8 Measurement of expression Mab (Dot Blot/ELISA)	3 days	Mon 3/15/10	Wed 3/17/10
9 Measurement of biological activity	4 days	Mon 3/15/10	Thu 3/18/10
10 B2b. Production of initial clones (rcTA)	58 days	Fri 2/26/10	Tue 5/18/10
11 Transfection	3 days	Fri 2/26/10	Tue 3/2/10
12 pool stabilization	10 days	Wed 3/3/10	Tue 3/16/10
13 Growth of selected 1000 clones	15 days	Wed 3/17/10	Tue 4/6/10
14 Induction & measurement of expression Mab (Dot Blot/ELISA)	6 days	Wed 4/7/10	Wed 4/14/10
15 Expansion of 50 best high-expressors	17 days	Thu 4/15/10	Fri 5/7/10
16 Induction & measurement of expression Mab (SDS-PAGE, Western)	7 days	Mon 5/10/10	Tue 5/18/10
17 Measurement of biological activity and/or Mass psec analy	5 days	Mon 5/10/10	Fri 5/14/10
18 B3. Selection of high producing clones	48 days	Mon 5/17/10	Wed 7/21/10
19 Sub-cloning of 20 clones (=150 - 200 total sub-clones)	2 days	Mon 5/17/10	Tue 5/18/10
20 Expansion of 150-200 sub-clones, back-up/storage	17 days	Wed 5/19/10	Thu 6/10/10
21 Induction & measurement of expression Mab (Dot Blot/ELISA)	6 days	Fri 6/11/10	Fri 6/18/10
22 Expansion of best 20 high-expressors	17 days	Mon 6/21/10	Tue 7/13/10
23 Measurement of expression Mab (SDS-PAGE/Western)	6 days	Wed 7/14/10	Wed 7/21/10
24 B4. Stability studies	62 days	Thu 7/22/10	Fri 10/15/10
25 Passaging of best 16 high-expressors	52 days	Thu 7/22/10	Fri 10/1/10
26 Induction & measurement of expression Mab (ELISA and/c	10 days	Mon 10/4/10	Fri 10/15/10
27 B5. Molecular characterization of the best clone	20 days	Thu 7/22/10	Wed 8/18/10

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Note: Certification for absence of Mycoplasma is included

Cost of cell line development:

- Approx. US\$ 160,000 for all of the aforementioned steps;

Options:

- Media optimization;
- Bioreactor process development;
- DSP process development.

Expression system license fees and royalties:

- US\$ 20 to 60,000 annually upon start of clinical trials depending on the expression system used;
- Maximum of US\$ 20,000 fees during R&D phase;

Royalties:

- 0.2-0.4%, depending on expression system used

References:

Mullick A. et. al., BMC Biotechnol., 2006 Nov 3; 6:43. The cumate gene-switch: a system for regulated expression in mammalian cells. [PMID: 17083727] (Free article in PMC at Journal site – <http://www.biomedcentral.com/1472-6750/6/43>).

Caron A. et al., BMC Biotechnol. 2009 May 11;9:42., Fluorescent labeling in semi-solid medium for selection of mammalian cells secreting high-levels of recombinant proteins. [PMID: 19432976]

About Viropro, Inc. (OTC BB- NASDAQ: VPRO):

Viropro, Inc., which conducts operations through its subsidiary Viropro International Inc., specializes in the transfer of its technologies for industrial production of biogeneric therapeutic proteins for the treatment of various diseases including cancer, diabetes, hepatitis or multiple sclerosis. The company's principal objective is to develop and provide high-yield, robust manufacturing process technologies and biopharmaceutical products for technology transfer to bio/pharmaceutical companies for global markets. (www.viropro.com)