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Stable cell line pools development report

I. PROJECT INFORMATION

Service Type: Stable Cell Line Development

Antibody Name: mAb1 (human IgG1 with Kappa light chains)

I.A. Antibody alias for the project: mAb1

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II. DESIGN OF THE FULL-LENGTH ANTIBODY FORMAT

> mAb1_HC protein

Confidential

> mAb1_LC protein

Confidential

III. GENE SYNTHESIS AND SUBCLONING

III.1 GENES CODING FOR THE TARGET PROTEINS

The cDNA coding for the target proteins described in the §2 will be chemically synthesized with optimization for the expression in CHO cells. The sequences are illustrated below.

> mAb1_HC cDNA

Confidential

> mAb1_LC cDNA

Confidential

III.2 SUBCLONING IN EXPRESSION VECTOR

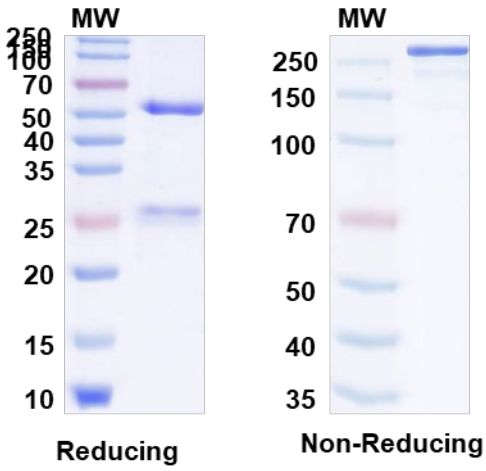
The cDNA sequences described in the §3.1 are subcloned in ProteoGenix' expression vector for mammalian stable cell lines development.

IV. SMALL-SCALE EXPRESSION TEST (TRANSIENT EXPRESSION)

IV.1 SHORT PROTOCOL DESCRIPTION

An endotoxin-free DNA preparation was done for the vector construction obtained as described in §3.2. By using the proprietary Xten transfection protocol, the plasmid was then transiently transfected in the CHO-K1 cells (30ml-culture). Culture medium was collected when the viability dropped the rAb was then purified on a protein A resin by using a standard method.

IV.2 RESULTS OF THE EXPRESSION AND PURIFICATION TESTS



Final sample QC. *Reducing and Non-Reducing PAGE analysis with Coomassie blue staining.*

MW. Molecular weight marker. 2µg per lane.

IV.3 CONCLUSION OF THE SMALL-SCALE PRODUCTION AND PURIFICATION TESTS

The yield and purity obtained are summarized in the Table 1.

Antibody	*Yield per liter* (mg/L)*	*Purity***
mAb1	250	>90%

Yield and purity obtained for the rAb purification tests.

**Extrapolated from the 30ml test.*

***Based on full length antibody observed on non-reduced SDS-PAGE.*

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V. STABLE CELL LINE POOL DEVELOPMENT

The vector construction obtained as described in §3 is used to develop stable CHO-K1 cell line pools expressing the target antibody. The selection process allowed to identify 5 mini-pools which were then amplified in 30mL fed-batch for production yield assessment.

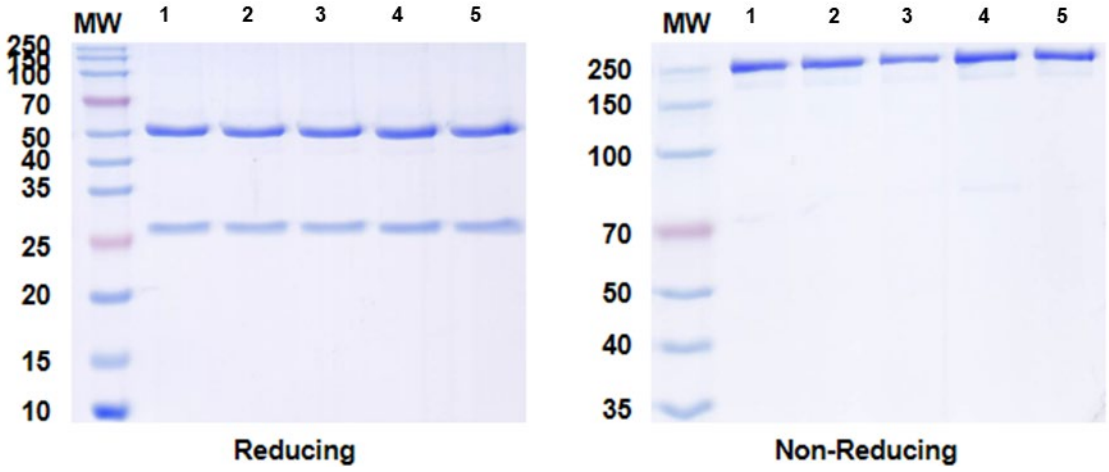
Pool name	*Yield (mg/L)**	Purity (%)**
Pool 1	***5 423***	***~100%***
Pool 2	***6 279***	***~100%***
Pool 3	***7 365***	***-***
Pool 4	***6 422***	***-***
Pool 5	***8 530***	***~100%***

Yield and purity obtained for the rAb purification tests.

**Obtained after purification for the 30ml-culture test.*

****Obtained by SEC-HPLC detailed in §6.*

Final buffer PBS, pH 7.5



Final sample QC. *Coomassie blue staining.*

Reducing and non-reducing PAGE analysis. 2ug per lane.

VI. SEC-HPLC ANALYSIS

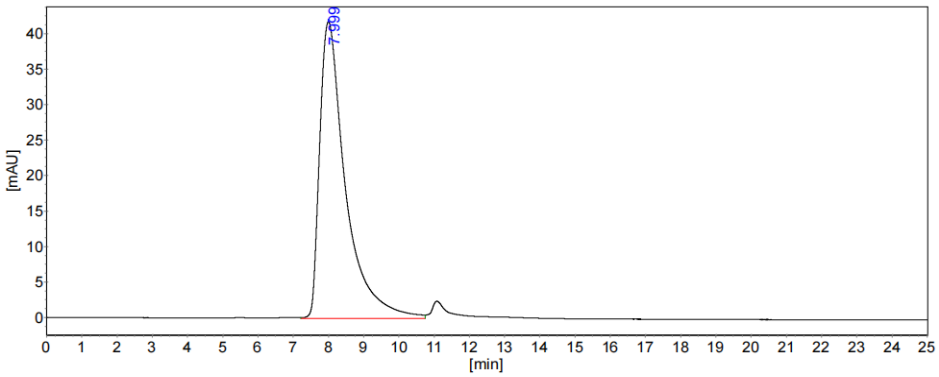
Starting from samples of each protein obtained after final conditioning and aliquoting as described in §5, SEC-HPLC analysis was performed to evaluate the purity level of mAb1 from stable pool 1, 2 and 5.

The analyses were performed with a HPLC Waters 2695 device equipped with a Photodiode Array Detector (2996).

VI.1 Results of the SEC-HPLC analysis

	Retention time (min)	***Area (µV/s)***	***Height (µV/s)***	***Purity (%)***
Pool 1	*7.999*	*2130154.4*	*41842.5*	*100.00%*
Pool 2	*8.045*	*1355778.5*	*8.045*	*100.00%*
Pool 5	*8.045*	*1421535.8*	*29693.9*	*100.00%*
SEC 300A Protein Standard	*5.999*	*170392.7*	*3508.1*	
	8.130	*244318.9*	*4303.0*	
	9.409	*155914.8*	*3147.6*	
	11.228	*150258.2*	*2444.9*	
	12.744	*118494.8*	*1658.0*	

Table 3. SEC-HPLC data



SEC-HPLC profile obtained for pool 1.

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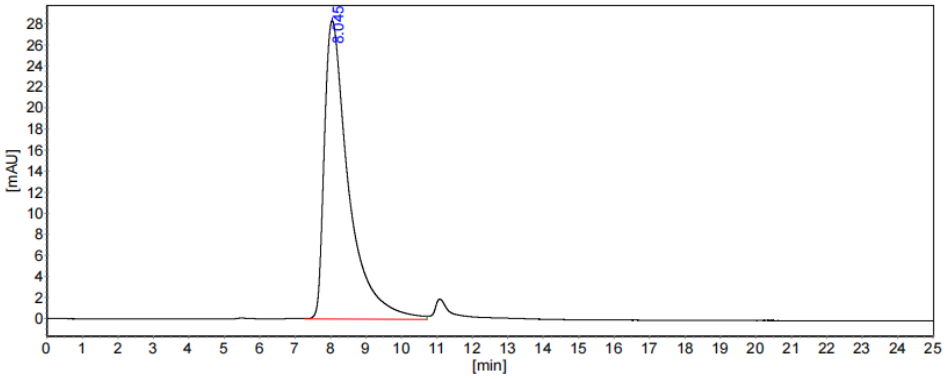
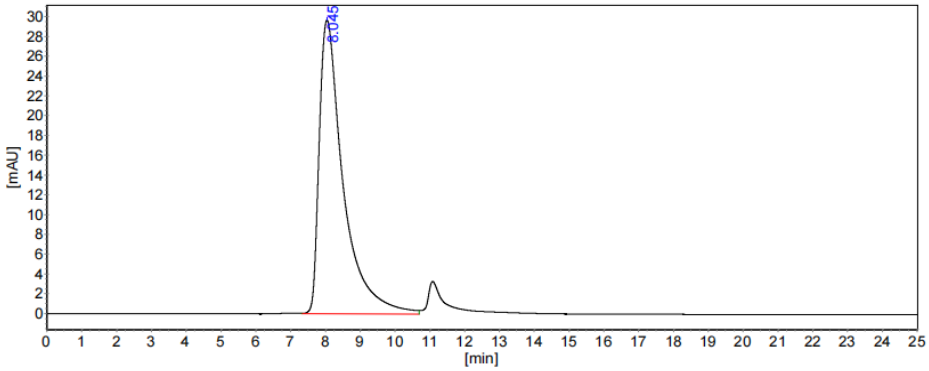
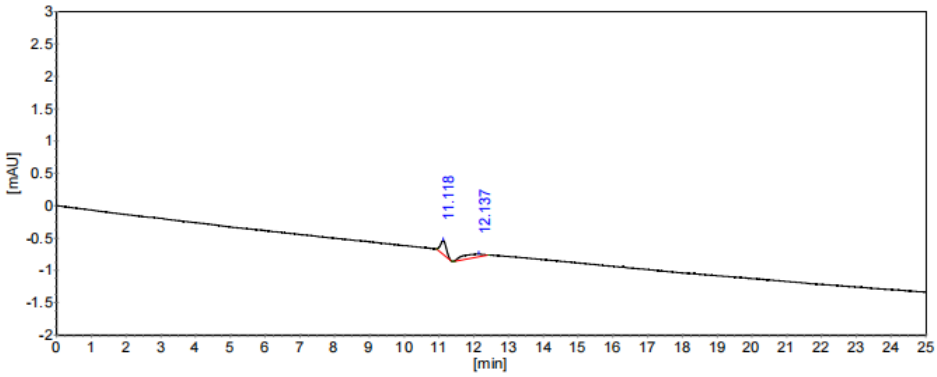


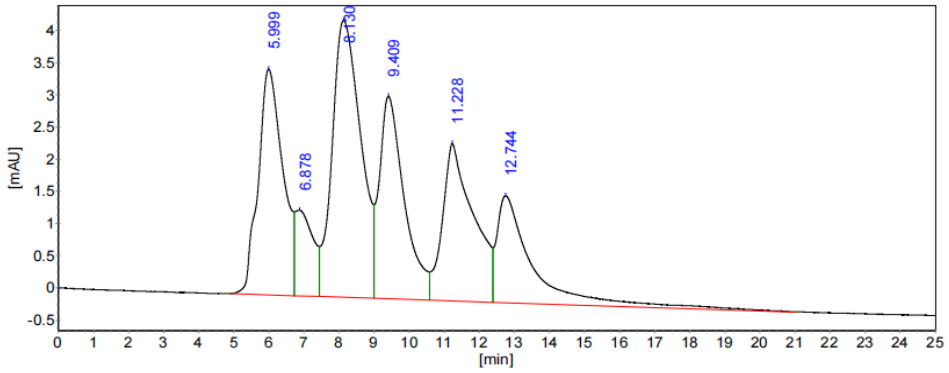
SEC-HPLC profile obtained for pool 2.



SEC-HPLC profile obtained for pool 5.



SEC-HPLC profile obtained for PBS.



SEC-HPLC profile obtained for Standard.

5.999. 670kDa. **8.130.** 150kDa. **9.409.** 45kDa. **11.228.** 17kDa. **12.744.** 1kDa.

VI.2 CONCLUSION OF THE SEC-HPLC ANALYSIS

The SEC-HPLC analyses indicate that all antibodies have a purity of 100% and exhibit no aggregation.

VII. PRODUCTION AND PURIFICATION SCALE-UP

Stable cell pool 5 was scale-up cultured and 1L fed-batch production was carried out in 3L shake flasks.

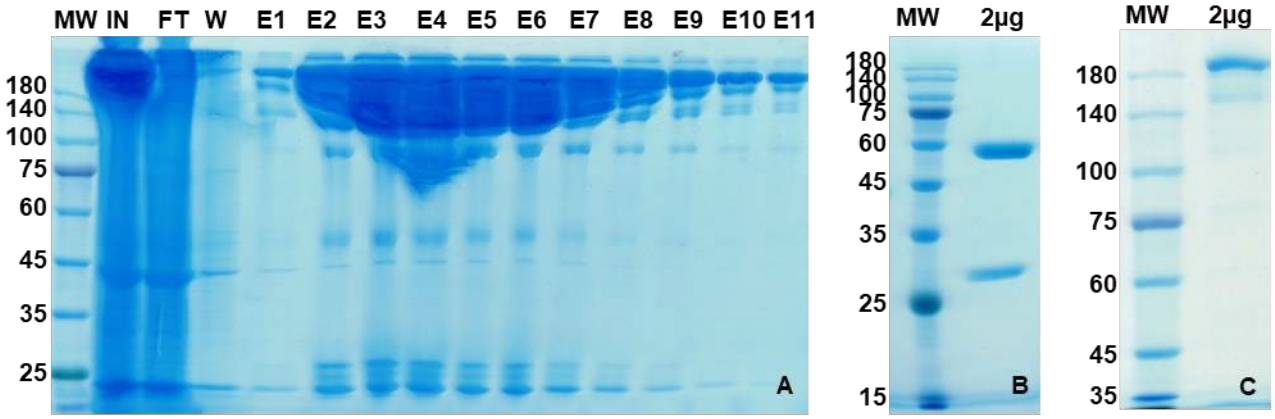
VII.1 CONCLUSION OF THE SCALE-UP PRODUCTION AND PURIFICATION TESTS

The yield and purity obtained from 1L scale-up are summarized below:

Antibody	*Quantity* (mg)*	*Purity***	*Endotoxin level (EU/mg)*
mAb1 from Stable pool_5	8 732	>95%	0.05

Yield and purity obtained for the mAb1 from Stable pool_5 production scale-up.

**Obtained after purification for the 1L culture. **Based on full length antibody observed on non-reduced SDS-PAGE. Final buffer PBS, pH 7.5.*



Purification of mAb1 from Stable pool 1. *Coomassie blue staining.*

A. Purification profile. **B and C.** Final sample QC (Pool of E1-E11)

A and C. Non-reducing PAGE analysis. **B.** Reducing PAGE analysis.

IN. Input sample. **FT.** Flow through. **W.** Wash step. **E1-E11.** Eluted fractions.