Production of recombinant antibodies for IVD applications

in stable cell pools and single cell clones

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Take-home messages

- Medix Biochemica applies methods used by biopharma to provide high-quality recombinant antibodies for diagnostic applications
- Two recombinant chimeric (RC; mouse-human) antibodies were developed based on mouse hybridomas mAbs
- The chimeric recombinant antibody products showed similar antigen-binding properties compared to the parental hybridoma mAb
- Recombinant antibodies produced by stable cell pools and single-cell clones showed comparable physico-chemical and functional characteristics

Introduction

Monoclonal antibodies (mAbs), used by the in-vitro diagnostic (IVD) industry, have traditionally been produced in mouse hybridomas, according to the Nobel Prize-recognised technique published in 1975[1]. Biotechnology company Medix Biochemica, was one of the pioneers in the commercial implementation of the method, and the company has been producing proprietary mAbs for the IVD industry since the 1980s. To complement the hybridoma technology, we have recently added recombinant technologies to our mAb development and manufacturing processes. These techniques will extend new antibody discovery options to multiple animal species and will enable antibody engineering for optimised performance.

In this study, we assessed the applicability of Horizon Discovery's stable glutamine synthetase knock-out CHO cell line (CHOSOURCE) for production of high-quality recombinant mAbs for IVD industry.

Materials and methods

- The variable regions of two hybridoma-derived MedixMAB-antibodies (anti-human NT-proBNP 1306 and anti-human cTnI 9701) were cloned into mammalian expression vector containing human IgG1κ constant regions
- After transfection, stable cell pools were initiated with $1x10^6$ viable cells and selection pressure of 25-75 µM methionine sulfoximine (MSX) was applied
- The best-producing cell pools were single cell cloned by limited dilution
- Parental stable pool and two single-cell clones of both cell lines were used for fed-batch antibody production
- The antibodies were purified from cell culture supernatants by protein A chromatography (MabSelect Sure resin, Cytiva)
- Physico-chemical properties of the purified antibodies were analysed by capillary isoelectric focusing (cIEF, iCE3, ProteinSimple), SEC (SEC BEH200Å 3.5µm 7.8 x 300mm, Waters) and capillary SDS-electrophoresis (PA800 Plus, Sciex)
- Functional characterisation of the antibodies was performed using biolayer interferometry (BLI, Octet RED96e, Sartorius) for kinetic parameters and an in-house fluorescent-immunoassay (FIA) for purified antigen and patient sample recognition.

Results

One stable cell pool and two single-cell clones were used to produce the recombinant versions of two hybridoma antibodies. The purified mAbs were analysed for charge heterogeneity, homogeneity, purity and immunoreactivity. We found only minor differences between the pool and single-cell clone products (Table 1, Fig. 1). The observed differences fall within the same limits as that we have previously observed with different batches of hybridoma mAbs.

When comparing the kinetics and immunoreactivities of recombinant chimeric versions to the original hybridoma mAbs, the performances were found similar (Table 1 and Fig. 2 and 3).

Discussion

Medix Biochemica is known for its high quality and batch-to-batch consistency of mAbs for diagnostic use. The ability to produce recombinant antibodies extends Medix Biochemica's toolkit for the design and development of novel high-quality antibodies for the IVD industry. In this study, Horizon Discovery's stable CHO-cell expression platform was found to be well-suited for the production of our chimeric recombinant antibodies.

The development of a stable cell line is lengthy as it requires clones to be isolated starting from single cells. In order to expedite our development process, we investigated the possibility to produce the first antibody batches using stable cell pools, which are available within 2-3 weeks from transfection. The observed variation between pool and single-cell clone products was minimal, as analysed by IEF-profile, homogeneity, purity and immunoreactivity. Similar results have been indicated for transient, minipool and stable cell clone products by others, too[2]. In the future, both stable pools and single-cell clones will be applied in our recombinant antibody development process.

Table 1. Purity and homogeneity of RC1306 and RC9701 lots purified from a stable cell pool or from two different single cell clones.

Analysis	RC1306 stable pool	RC1306 single cell clone no 1	RC1306 single cell clone no 2	RC9701 stable pool	RC9701 single cell clone no 1	RC9701 single cell clone no 2
Homogeneity	100%	100%	99%	100%	100%	100%
Purity	≥ 95%	≥ 95%	≥ 95%	≥ 95%	≥ 95%	≥ 95%

Table 2. Kinetics of the recombinant antibodies and the parental hybridoma mAbs

Product	ka (k-on) (1/Ms)	kd (k-off) (1/s)	KD (M)
1306	5.1×10^5	5.5×10^{-4}	1.7×10^{-9}
RC1306	2.3×10^5	4.7×10^{-4}	3.8×10^{-9}
9701	3.4×10^5	3.0×10^{-4}	1.2×10^{-9}
RC9701	2.5×10^5	3.3×10^{-4}	1.3×10^{-9}

Fig. 1. Overlay of A) IEF and B) immunoreactivity profiles of RC1306 antibody purified from a stable cell pool or from two different single cell clones. Similar data was found for RC9701.

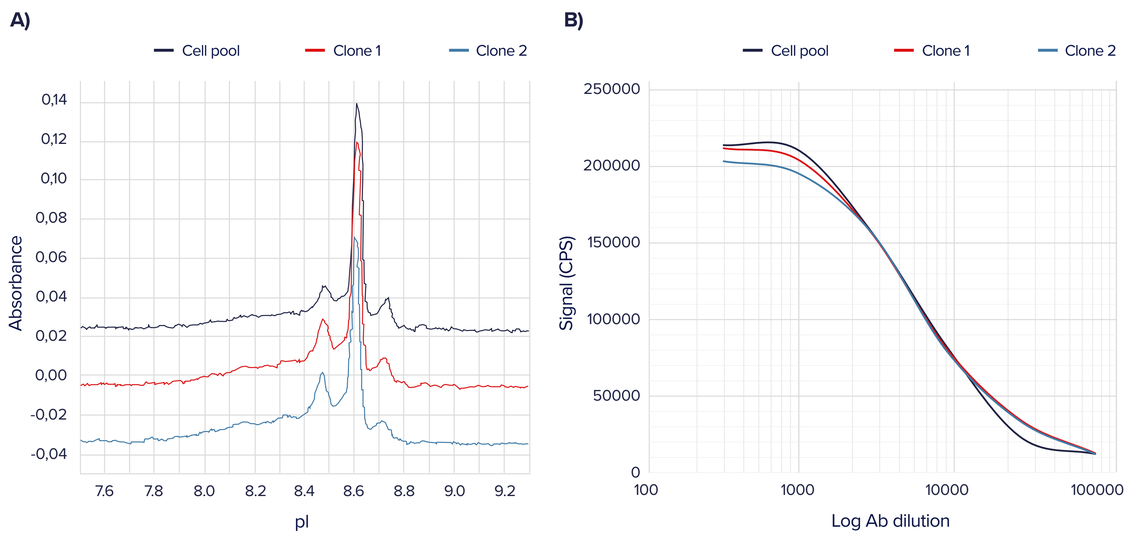
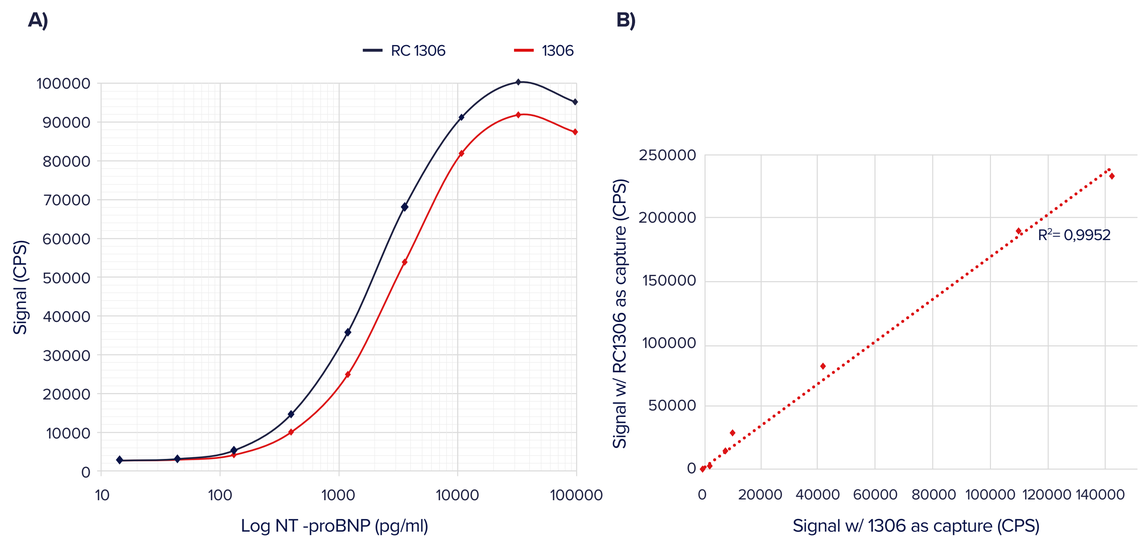


Fig. 2. A) Overlay of NT-proBNP standard curves from sandwich FIA using either recombinant RC1306 or hybridoma mAb 1306 as coating antibody paired with an Eu-labelled hybridoma mAb (anti-human NT-proBNP 1309) for detection. Similar data was found for RC9701. B) The performance of RC1306 was similar to the parental 1306 in detecting native NT-proBNP in human serum samples when paired with mAb 1309.



References
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