

domains were synthesized de novo and cloned into vectors pEE-HC, pEE-CK (IgG1 format) at Sall/NheI and Sall/BsiW1 restriction sites, respectively (Fig. 1, 2). The schematic representation of the IgG1 format is given in Fig. 3.

The resulting genetic constructs were used to transform CHO-T cell line. Proteins were isolated and purified according to standard methods by affinity chromatography on bacterial Protein A as described in Example 1. Electrophoresis was performed in denaturing 7.5% PAGE. The production performance of 22 candidates was below the threshold level (50 mg/l); therefore, they were not isolated and purified.

Example 7. Sequencing of high affinity clones

Variable domain genes of positive clones were sequenced according to standard protocols on Applied Biosystems 3130 Genetic Analyzer (Applied Biosystems) and analyzed.

Example 8. Determination of affinity of full-length antibodies on Forte Bio Octet RED 384

KD values of the resulting full-length candidates were determined on Forte Bio Octet RED 384.

SAX biosensors and biotin-modified CD20 peptide (Sigma Aldrich) were used for the study. Antibody Rituximab was used as a control. SAX biosensors were steeped into a solution containing biotinylated CD20 peptide at a concentration of 20 µg/ml, where the peptide was immobilized. Further analysis was conducted at 30°C using PBS containing 0.1% Tween 20 and 0.1% BSA as a working buffer.

After baseline recording in buffer solution, the sensors were immersed into wells containing antibody solution at a concentration of 10 µg/ml for 150 seconds, where the complex was associated. Complex dissociation in buffer solution was then detected for 300 seconds.

Binding curves, after subtracting a reference signal, were analyzed using Octet Data Analysis software (Version 9.0) in accordance with the standard procedure using 1:1 interaction model.

116 candidates were transferred for analysis. 67 out of them did not show any binding to peptide. The remaining 49 candidates interacted with peptide with nanomolar and micromolar affinity (Fig. 7 and Table 1).

Table 1

C andidate	Resp onse (nm)	kD (M)	kon (1/Ms)	kdis (1/s)	Full R ²
B CD132L -001	No binding	No binding	No binding	No binding	No binding
B CD132L -002	No binding	No binding	No binding	No binding	No binding
B CD132L -003	0.25 76	1.87E- 07	3.06E +05	5.73E -02	0.990 9
B	No	No	No	No	No