Objective: Increasing productivity of antibody fragment purification

Fab (b)
3
Corporation,
: antibody fragments containing Nishihachijo Fab (B)

Acknowledgements
(z)

Fab (A) Fab (B) Fab (C)

In vitro affinity maturation of SpG

Wild type Protein G and Protein L need to modify their affinity as ligands for capture chromatography resin

Enhanced binding capacity of KNK SpG Resin, KanCap™ G

KanCap™ G could capture wide range of antibody fragments containing kappa light chain as commercial PpL resin could hardly bind.

Low affinity to almost all of CH1

Figure 1. Characteristics of antibody ligands for antibody fragments

Figure 2. In vitro affinity maturation by using a ribosome display system, PUREflex®RD

The association constant of selected SpG mutants increased from 30 to 80-fold compared to wild type SpG.

Figure 3. Association constant (Kd) to Fab's of SpG mutants selected by in vitro affinity maturation

KanCap™G showed higher binding capacity for all types of human Fabs than commercial SpG resins and no leakage in washing step.

Figure 4. Chromatographic analysis of binding capacity of SpG resins

Dynamic binding capacity (DBC) at 5% breakthrough was measured at a residence time of 4 min.

KanCap™G: SpG mutant selected by affinity maturation was immobilized to cellulose base matrix.

Fab (A) Fab (B) Fab (C) Fab (A)Fab (B)Fab (C)

Figure 5. SDS-PAGE analysis of Fab purification using KanCap™ G

KanCap™ G could capture both types of Fabs, kappa and lambda type and eliminate byproduct (e.g. LC monomer, LC dimer) from Fab expressed in Yeast Sup.

High recovery and specificity in Fab purification

High recovery and purity in Fab purification

KanCap™G and PpL agarose (A) were immobilized to cellulose base matrix.

KanCap™ L could capture wide range of antibody fragments containing kappa light chain as commercial PpL resin could hardly bind.

Figure 6. Schematic representation of the PpL/Fab complex and surface analysis (generated by Pymol).

KanCap™ L could capture wide range of antibody fragments containing kappa light chain as commercial PpL resin could hardly bind.

Figure 7. The difference of binding spectra between wild type and improved Protein L

KanCap™ L could capture wide range of antibody fragments containing kappa light chain as commercial PpL resin could hardly bind.

Figure 8. SDS-PAGE analysis of Fab purification samples using KanCap™ L

Fab was highly purified with high yield from E.coli lysate only by one-step chromatography using KanCap™ L.

Conclusions

KanCap™ G has high binding capacity and recovery for all types of human Fabs.

KanCap™ L has wide binding spectra and high binding capacity for the antibody fragments containing kappa light chain.

Kaneka proposes new purification platform process for antibody fragments using our novel Protein G and Protein L chromatography resin.

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