**Supplementary data 1**

**Mathematical algorithm for IP-HPLC analysis**

- IP-HPLC peak area (mAU\*s) contains antigen peak ({Ag}), antibody peak ({Ab}), and antigen-antibody complex peak ({Ag-Ab}).

A (mAU\*s) = {Ag1} + {Ab1} + {Ag2-Ab2}

- UV spectrum shows relatively proportional value (α) between ({Ag} + {Ab}) and {Ag-Ab}; {Ag-Ab} = α({Ag} + {Ab})

A (mAU\*s) = {Ag1} + {Ab1} + α({Ag2} + {Ab2})

A (mAU\*s) = ({Ag1} + α({Ag2}) +({Ab1} + α{Ab2})

{Ag1} + α({Ag2} (mAU\*s) = A - ({Ab1} + α{Ab2})

- When the antibody was monospecific or monoclonal to antigen, Ag1/Ag2 = Ab1/Ab2 = β.

{Ag1} + $\frac{α}{β}$({Ag2} (mAU\*s) = A - ({Ab1} + α{Ab2})

{Ag1} (1 + $\frac{α}{β}$) (mAU\*s) = A - ({Ab1} + α{Ab2})

{Ag1} (mAU\*s) = $\frac{β(A - \left(\left\{Ab1\right\}+ α\left\{Ab2\right\}\right))}{α+β}$

And

{Ag1} + α({Ag2} (mAU\*s) = A - ({Ab1} + α{Ab2})

β({Ag2} + α({Ag2} (mAU\*s) = A - ({Ab1} + α{Ab2})

β({Ag2} + α({Ag2} (mAU\*s) = A - ({Ab1} + α{Ab2})

{Ag2}(α + β) (mAU\*s) = A - ({Ab1} + α{Ab2})

{Ag2}(α + β) (mAU\*s) = $\frac{A - (\{Ab1\} + α\{Ab2\})}{α + β}$

* And then, the objective antigen expression was {Ag1} + {Ag2}.

{Ag1} + {Ag2} (mAU\*s) = $\frac{\left(1+β\right)A-2(\left\{Ab1\right\}+ α\left\{Ab1\right\})}{α + β}$

- {Ag1c} + {Ag2c} (mAU\*s) is an objective antigen expression of control group, while {Ag1e} + {Ag2e} (mAU\*s) is an objective antigen expression of experimental group.

- The ratio compared between experiment and control objective antigen expression is ({Ag1e} + {Ag2e})/({Ag1c} + {Ag2c}).

$\frac{\{Ag1e\} + \{Ag2e\}}{\{Ag1c\} + \{Ag2c\}}$ = $\frac{Ae\left(1+β\right) - 2(\left\{Ab1e\right\} + α\left\{Ab2e\right\})}{Ac\left(1+β\right) - 2(\left\{Ab1c\right\} + α\left\{Ab2c\right\})}$

 = $\frac{Ae - \frac{2(\left\{Ab1e\right\} + α\left\{Ab2e\right\}}{1 + β}}{Ac - \frac{2(\left\{Ab1c\right\} + α\left\{Ab2c\right\}}{1 + β}}$

- $\frac{2(\left\{Ab1e\right\} + α\left\{Ab2e\right\}}{1 + β}$ and $\frac{2(\left\{Ab1c\right\} + α\left\{Ab2c\right\}}{1 + β}$ are replaceable with γAave (Aave is average of Ac and Ae).

- Therefore,

$\frac{\{Ag1e\} + \{Ag2e\}}{\{Ag1c\} + \{Ag2c\}}$ = $\frac{Ae - γAave}{Ac - γAave}$

- Because ({Ag1e} + {Ag2e}) and ({Ag1c} + {Ag2c}) are mathematically hypothetical value (mAU\*s), their square root value may approximate the comparable expression level (mAU).

$\sqrt{\frac{\{Ag1e\} + \{Ag2e\}}{\{Ag1c\} + \{Ag2c\}}}$ = $\sqrt{\frac{Ae - γAave}{Ac - γAave}}$

$\frac{Experiment antigen expression level}{Control antigen expression level} $ = $\sqrt{\frac{Ae - γAave}{Ac - γAave}}$ x 100 (%)

- γ can be determined by experimental IP-HPLC. If 15%-reduced amount of objective protein sample was applied to Protein A/G bead column compared to control group. The IP-HPLC results were as follow;



When the eluted proteins containing antibody were analyzed with 30 cm long column at low running speed (0.3 mL/min), the proteins were slightly separated but still appeared much overlapped in chromatography.

- And then,

$\frac{Experiment antigen expression level}{Control antigen expression level} $ = $\sqrt{\frac{Ae - γAave}{Ac - γAave}}$ x 100 = 85 (%)

$\sqrt{\frac{Ae - γAave}{Ac - γAave}}$ x 100 = 85 (%)

$\sqrt{\frac{539.658 - 567.209γ}{594.76 - 567.209γ}}$ x 100 = 85 (%)

Therefore, constant γ can be calculated as 0.6985, and used to subtract other elements besides objective protein.

From this algorithm, the relative ratio (%) between objective protein level and control protein level can be obtained, albeit it is impossible to get the concentration of objective protein through IP-HPLC.