Supplementary Materials for

RSM-based Model to Predict Optimum Fermentation Conditions for Soluble Expression of the Antibody Fragment Derived from 4D5MOC-B Humanized Mab in SHuffle™ T7 E. coli

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Tables S1 and S2
Figure S1
Table S1. ANOVA quadratic model for soluble fraction.

| Source         | Sum of Squares | df | Mean Square | F-value | P-value | P-value | Significant |
|----------------|----------------|----|-------------|---------|---------|---------|-------------|
| Model          | 2.253E+13      | 9  | 2.504E+12   | 48.73   | 0.0002  |         | significant |
| A-IPTG         | 4.664E+12      | 1  | 4.664E+12   | 90.78   | 0.0002  |         |             |
| B-OD           | 6.315E+11      | 1  | 6.315E+11   | 12.29   | 0.0172  |         |             |
| C-Temperature  | 5.697E+12      | 1  | 5.697E+12   | 110.88  | 0.0001  |         |             |
| AB             | 9.079E+12      | 1  | 9.079E+12   | 176.70  | < 0.0001|         |             |
| AC             | 6.546E+11      | 1  | 6.546E+11   | 12.74   | 0.0161  |         |             |
| BC             | 7.833E+11      | 1  | 7.833E+11   | 15.24   | 0.0114  |         |             |
| A²             | 6.209E+11      | 1  | 6.209E+11   | 12.09   | 0.0177  |         |             |
| B²             | 6.599E+10      | 1  | 6.599E+10   | 1.28    | 0.3085  |         |             |
| C²             | 2.591E+11      | 1  | 2.591E+11   | 5.04    | 0.0747  |         |             |
| Residual       | 2.569E+11      | 5  | 5.138E+10   |         |         |         |             |
| Lack of Fit    | 6.059E+10      | 3  | 2.020E+10   | 0.2058  | 0.8855  |         | not significant |
| Pure Error     | 1.963E+11      | 2  | 9.815E+10   |         |         |         |             |
| Cor Total      | 2.279E+13      | 14 |             |         |         |         |             |
Table S2. ANOVA quadratic model for Total Expression.

| Source       | Sum of Squares | df | Mean Square | F-value | P-value | P-value Status |
|--------------|----------------|----|-------------|---------|---------|----------------|
| Model        | 7.789E+05      | 9  | 86539.90    | 33.66   | 0.0006  | significant    |
| A-IPTG       | 89057.03       | 1  | 89057.03    | 34.64   | 0.0020  |                |
| B-OD         | 18783.62       | 1  | 18783.62    | 7.31    | 0.0426  |                |
| C-Temperature| 3.062E+05      | 1  | 3.062E+05   | 119.11  | 0.0001  |                |
| AB           | 3.266E+05      | 1  | 3.266E+05   | 127.04  | < 0.0001|                |
| AC           | 4039.67        | 1  | 4039.67     | 1.57    | 0.2654  |                |
| BC           | 15706.51       | 1  | 15706.51    | 6.11    | 0.0564  |                |
| A²           | 3429.81        | 1  | 3429.81     | 1.33    | 0.3003  |                |
| B²           | 11959.57       | 1  | 11959.57    | 4.65    | 0.0835  |                |
| C²           | 2289.39        | 1  | 2289.39     | 0.8905  | 0.3887  |                |
| Residual     | 12854.66       | 5  | 2570.93     |         |         |                |
| Lack of Fit  | 6135.03        | 3  | 2045.01     | 0.6087  | 0.6703  | not significant|
| Pure Error   | 6719.63        | 2  | 3359.82     |         |         |                |
| Cor Total    | 7.917E+05      | 14 |             |         |         |                |
Figure S1. SDS-PAGE analysis of antiEpEX-scFv expression in different experiments according to Box–Behnken Design. M: Protein molecular weight markers, 1–15: experimental conditions, (IS: insoluble fraction; S: soluble fraction; T: Total cell lysate). (C+): induced total lysate of *E. coli* SHuffle (DE3) in LB medium. (arrow): recombinant antiEpEX-scFv protein [~ 30 kDa].