

## **9. Selection of phage clones displaying HuScFv that bound to proteins in P1, P3, P4, P5, and P8 from human antibody phage display library**

The venom protein components, *i.e.*, lethal natrin and long neurotoxin (P4 and P8, respectively), cobra venom factor (P1) and phospholipase A2 isoenzymes (P3 and P5), were individually used to select HuScFv specific phage clones from the so-constructed antibody phage display library. Phage library ( $\sim 10^{11}$  phage particles) were added into each wells of ELISA plate coated with individual protein fractions (P1, P3, P4, P5 or P8) as described in **Section 9.2.2 of Chapter IV**. Single round of bio-panning was performed for P1, P3, and P5. Both single and triple rounds of bio-panning were performed for P4 and P8. **Table 14** concludes the results of the bio-panning with protein components of the Thai cobra venom.

### **9.1 Single round of bio-panning with cobra venom factor (P1)**

A total of 252 cfu of HB2151 *E. coli* colonies were obtained. Initially, 11 (4%) were screened for the presence of *huscFv* and ability of the clones to express HuScFv. The remaining colonies were scraped from the plate and kept as a glycerol stock at  $-80^{\circ}\text{C}$ . Among the 11 selected clones, 10 clones (91%, **Figure 59**) contained *huscFv* as shown at size  $\sim 1,000$  bp and 8 clones (80%, **Figure 60**) could express HuScFv which appeared as a single or double band(s) or both at  $M_r \sim 25-27$  kDa.

### **9.2 Single round of bio-panning with P3 which predominantly contained the phospholipase**

A total of 38 cfu of HB2151 *E. coli* colonies were obtained. All of the 38 colonies (100%) were screened for *huscFv* and HuScFv expression. Among them, only 20 colonies (53%, **Figure 61**) contained *huscFv* seen as a band at  $\sim 1,000$  bp and only 8 colonies (40%, **Figure 62**) could express HuScFv seen as band(s) at  $M_r \sim 25-27$  kDa.

### **9.3 Single round of bio-panning with P4 which contained predominantly natrin toxin**

A total of 22 cfu of HB2151 *E. coli* colonies were obtained. All of the 22 colonies (100%) were screened for *huscFv* and HuScFv expression. Among the 22 colonies, only 12 colonies (55%, **Figure 63**) contained *huscFv* seen at the size ~1,000 bp. Among the 12 colonies, only 9 colonies (75%, **Figure 64**) could express HuScFv seen at *Mr* ~25-27 kDa.

### **9.4 Triple rounds of bio-panning with P4 (predominantly natrin)**

A total of 18 cfu of HB2151 *E. coli* colonies were obtained. All of the 18 colonies (100%) were screened for *huscFv* and HuScFv expression. Among them, 7 colonies (39%, **Figure 65**) contained *huscFv* as shown at the size ~1,000 bp and 4 clones (57%, **Figure 66**) could express HuScFv seen as band(s) at *Mr* ~ 25-27 kDa.

### **9.5 Single round of bio-panning with P5 which mainly contained phospholipase**

A total of 327 cfu of HB2151 *E. coli* colonies were obtained. Only 20 colonies (6%) were screened for *huscFv* and HuScFv expression and the remained colonies were kept as a pool in glycerol at -80°C. Among the 20 colonies, 19 clones (95%, **Figure 67**) contained *huscFv* as shown at the size ~1,000 bp and all clones (100%, **Figure 68**) could express HuScFv (*Mr* ~25-27 kDa).

### **9.6 Single round of bio-panning with P8 which predominantly contained principally the long neurotoxin**

The single round of bio-panning using P8 as an immobilized antigen was performed two times. For the first time, a total 26 cfu were obtained. All colonies (100%) were screened for *huscFv* and HuScFv expression. Among 26 colonies, 21 colonies (84%, **Figure 69**) contained *huscFv* as shown at size ~1,000 bp and only 10 colonies (48%, **Figure 70**) could express HuScFv (*Mr* ~25-27 kDa).

For the second time of the single round bio-panning, a total 20 cfu of HB2151 *E. coli* colonies were obtained. All colonies (100%) were screened for *huscFv* and HuScFv expression. Among them, 12 colonies (60%, **Figure 71**) contained *huscFv* as shown at the size of ~1,000 bp and only 9 colonies (75%, **Figure 72**) could express HuScFv (*Mr* ~25-27 kDa).

### **9.7 Triple rounds of bio-panning with P8 (principally contained long neurotoxin)**

A total of 31 cfu of HB2151 *E. coli* colonies were obtained. All colonies (100%) were screened for *huscFv* and HuScFv expression. Among them, 12 colonies (39%, **Figure 73**) contained *huscFv* as shown at the size of ~1,000 bp and only 7 colonies (58%, **Figure 74**) could express HuScFv. The size of the soluble HuScFv was ~25-27 kDa.

**Table 14** Results of phage bio-panning using P1, P3, P4, P5, and P8 as antigens

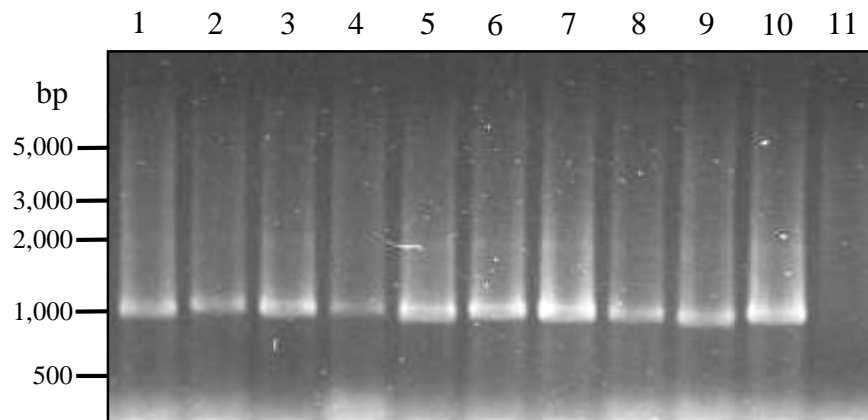
<b>Antigen</b>	<b>Panning method</b>	<b>No. of <i>E. coli</i> colonies</b>	<b>Number of screened clones (%)</b>	<b>Number of clones carrying <i>huscFv</i> (%)</b>	<b>Number of clones expressing HuScFv (%)</b>
P1	single round	252	11 (4)	10 (91)	8 (80)
P3	single round	38	38 (100)	20 (53)	8 (40)
P4	single round	22	22 (100)	12 (54)	9 (75)
	three rounds	18**	18 (100)	7 (39)	4 (57)
P5	single round	327	20 (6)	19 (95)	19 (100)
P8*	single round	25	25 (100)	21 (84)	10 (48)
	(1 <sup>st</sup> time)				
	single round	20	20 (100)	12 (60)	9 (75)
	(2 <sup>nd</sup> time)				
	three rounds	31**	31 (100)	12 (39)	7 (58)

\* Single round of bio-panning was performed twice; and the three rounds of bio-panning was performed once.

\*\* Transformed *E. coli* clones were prepared by adding HB2151 *E. coli* cells directly into wells of bound phages after the unbound phages had been washed away.

*huscFv*, gene encoding human single chain variable fragment of antibody

HuScFv, human single chain variable fragment of antibody

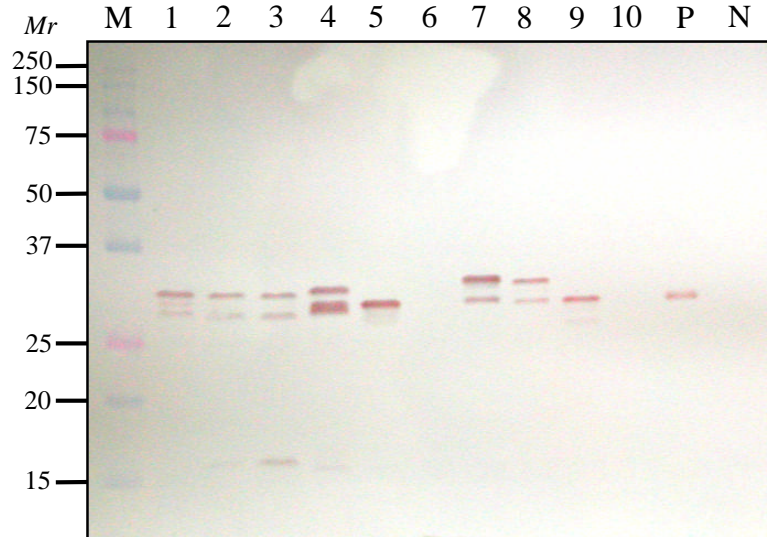


**Figure 59** Amplicons of *huscFv* of the 11 randomly selected transformed HB2151 *E. coli* colonies from a total of 252 transformed HB2151 *E. coli* colonies infected with phages that bound to **P1** from the single round of bio-panning.

Lanes 1-10, the *huscFv* positive HB2151 *E. coli* clones no. 1-10, respectively

Lane 11, negative *huscFv* *E. coli* colonies

Numbers at the left are DNA sizes in bp



**Figure 60** Results of HuScFv expressions and Western blot analysis of 10 *huscFv*-pCANTAB5E-transformed HB2151 *E. coli* colonies derived from single round of bio-panning with **P1** which predominantly contained the cobra venom factor.

Lane M, standard protein markers

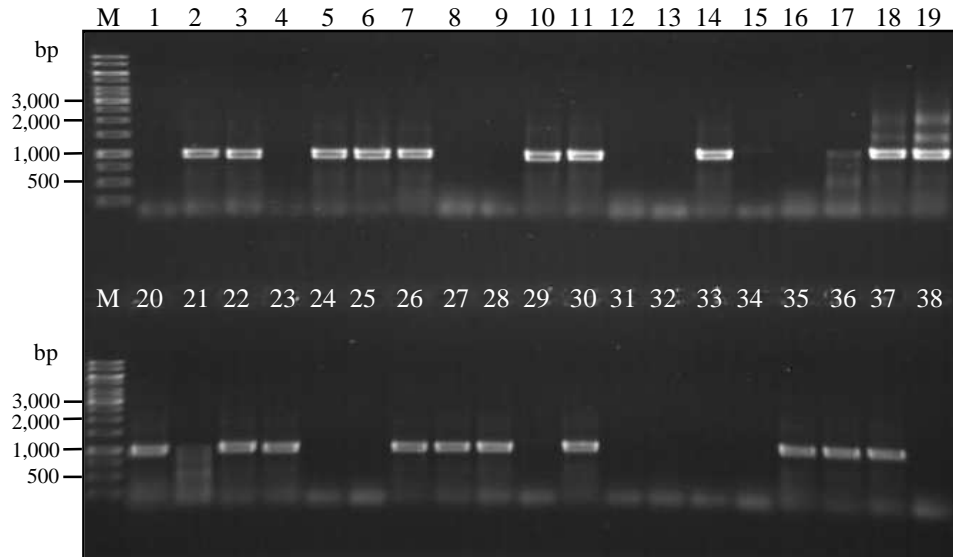
Lanes 1-10, whole cell lysates of HB2151 *E. coli* clones no. 1-10

Lane P, whole cell lysate of HB2151 *E. coli* harboring mouse *scFv*-phagemid vector (positive control)

Lane N, whole cell lysate of HB2151 *E. coli* harboring pUC19 (negative control)

Numbers at the left of the figure are relative molecular masses (*Mr*)

\*Some HuScFv appeared as multiple protein bands because the HuScFv may be mature (without signal peptide; lower band of the doublet) or immature (with signal peptide; upper band of the doublet) and some proteins were degraded.

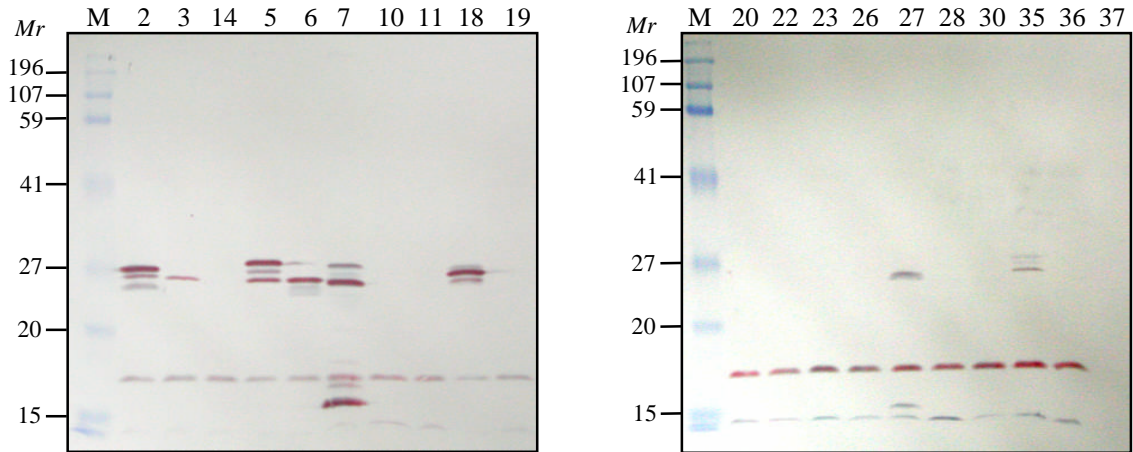


**Figure 61** Results of *huscFv* amplification of the 38 transformed HB2151 *E. coli* clones selected from single round of bio-panning with **P3** which principally contained phospholipase A2.

Lanes M, GeneRuler 1 kb DNA ladder

Lanes 1-38, the *huscFv* amplicons of HB2151 *E. coli* clones no. 1-38, respectively

Numbers at the left of the figure are DNA sizes in bp



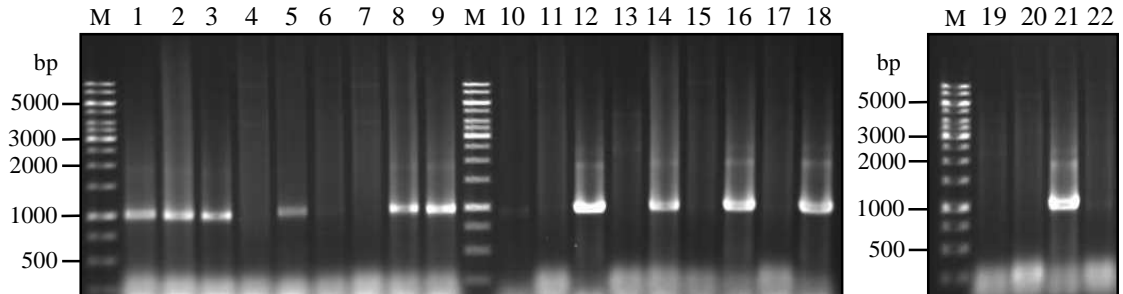
**Figure 62** Results of HuScFv expression and Western blot analysis of the 20 *huscFv*-pCANTAB5E-transformed HB2151 *E. coli* colonies derived from single round of bio-panning with **P3**.

Lanes M, standard protein markers

Whole cell lysates of eight HB2151 *E. coli* colonies from the 20 colonies (40%) of *huscFv*-carrying HB2151 *E. coli* (lanes 2, 3, 5-7, 10, 11, 18-20, 22, 23, 26-28, 30 and 35-37 of **Figure 61**) could express HuScFv at relative molecular masses (*Mr*) ~25-27 kDa (lanes 2, 3, 5-7, 18, 27, and 35).

Numbers at the left of both blocks of the figure are relative molecular masses (*Mr*).

\*Some HuScFv appeared as multiple protein bands because the HuScFv may be mature (without signal peptide) or immature (with signal peptide) and some proteins were degraded.

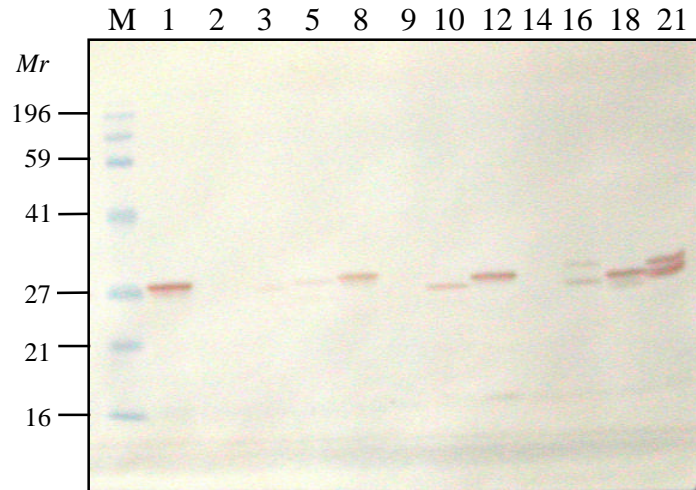


**Figure 63** Results of *huscFv* amplification of the 22 transformed HB2151 *E. coli* clones selected from single round of bio-panning with **P4** which principally contained natrin toxin.

Lanes M, GeneRuler 1 kb DNA ladder

Lanes 1-22, the *huscFv* amplicons of the transformed HB2151 *E. coli* clones no. 1-22, respectively

Numbers at the left of both blocks of the figure are DNA sizes in bp



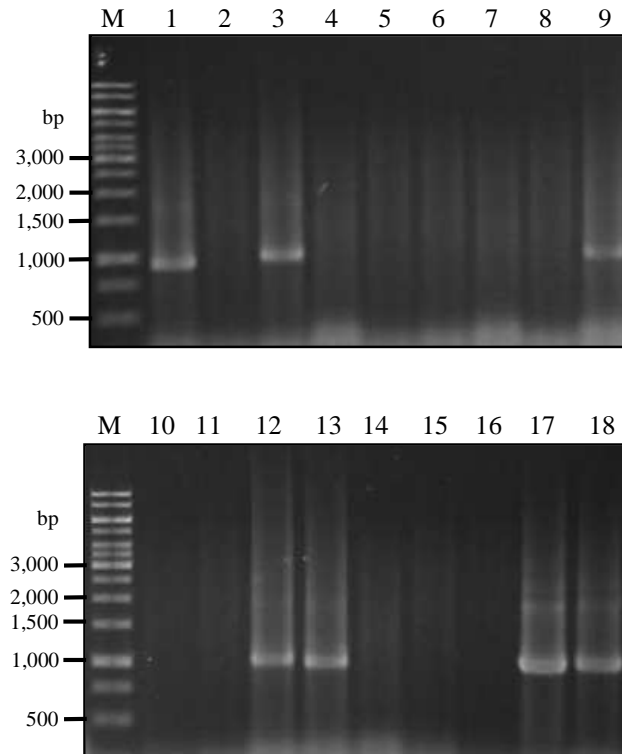
**Figure 64** Results of HuScFv expression and Western blot analysis of the 12 *huscFv*-pCANTAB5E-transformed HB2151 *E. coli* colonies derived from single round of bio-panning with **P4**.

Lane M, standard protein markers

Whole cell lysates of nine HB2151 *E. coli* colonies from 12 colonies (75%) of *huscFv*-carrying HB2151 *E. coli* colonies (lanes 1-3, 5, 8, 9, 10, 12, 14, 16, 18, and 21 of **Figure 63**) could express HuScFv at relative molecular masses (*Mr*) ~25-27 (lanes 1, 3, 5, 8, 10, 12, 16, 18, and 21).

Numbers at the left of the figure are relative molecular masses (*Mr*).

\*Some HuScFv appeared as multiple protein bands because the HuScFv may be mature (without signal peptide) or immature (with signal peptide) and some proteins were degraded.

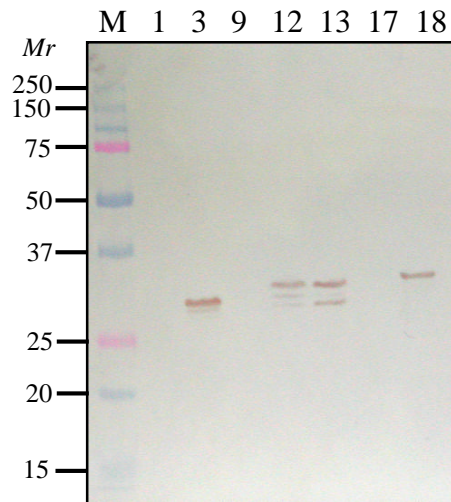


**Figure 65** Results of *huscFv* amplification of the 18 transformed HB2151 *E. coli* colonies derived from the third round of bio-panning with **P4** (principally contained natrin).

Lanes M, GeneRuler 1 kb DNA ladder

Lanes 1-18, the *huscFv* amplicons of HB2151 *E. coli* clones no. 1-22, respectively.

Numbers at the left of both blocks of the figure are DNA sizes in bp



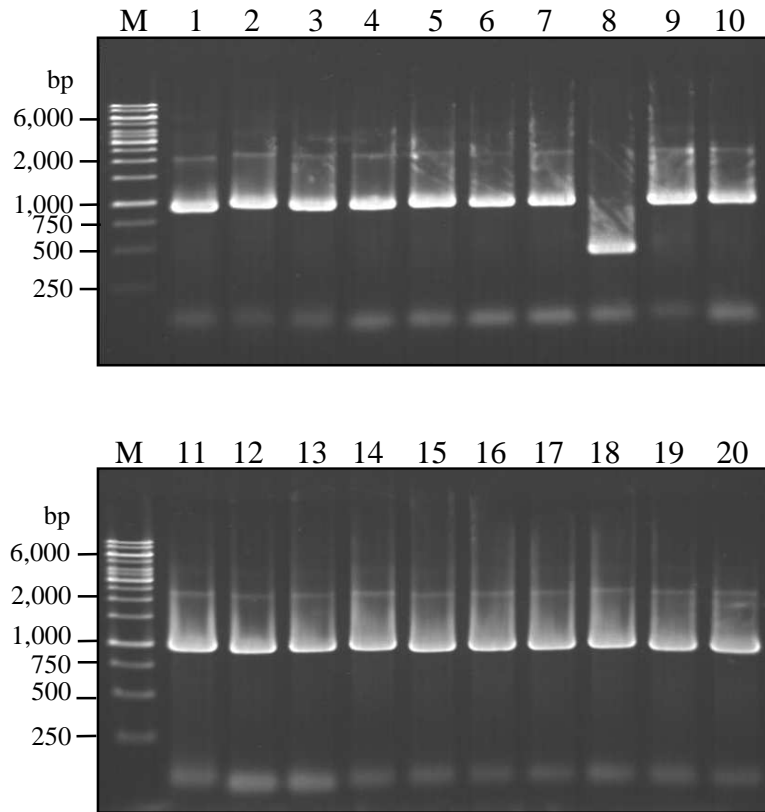
**Figure 66** Results of HuScFv expression and Western blot analysis of the 7 *huscFv*-pCANTAB5E-transformed HB2151 *E. coli* colonies derived from the third round of bio-panning with **P4**.

Lane M, standard protein markers

Whole cell lysates of the 4 transformed HB2151 *E. coli* colonies from 7 colonies (57%) of *huscFv*-carrying HB2151-*E. coli* colonies (lanes 1, 3, 9, 12, 13, 17, and 18 of **Figure 65**) could express HuScFv at relative molecular masses (*Mr*) ~25-27 (lanes 3, 12, 13, and 18).

Numbers at the left of the figure are relative molecular mass (*Mr*).

\*Some HuScFv appeared as multiple protein bands because the HuScFv may be mature (without signal peptide) or immature (with signal peptide) and some proteins were degraded.

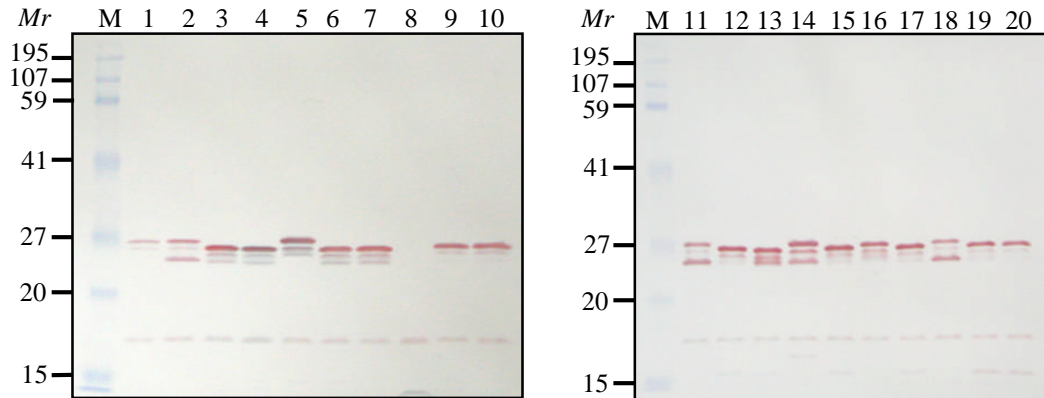


**Figure 67** Results of the *huscFv* amplification of the 20 transformed HB2151 *E. coli* colonies derived from the single round of bio-panning with **P5** which principally contained phospholipase A2.

Lanes M, GeneRuler 1 kb DNA ladder

Lanes 1-20, the *huscFv* amplicons of HB2151 *E. coli* clones no. 1-20, respectively

Numbers at the left of both blocks of the figure are DNA sizes in bp



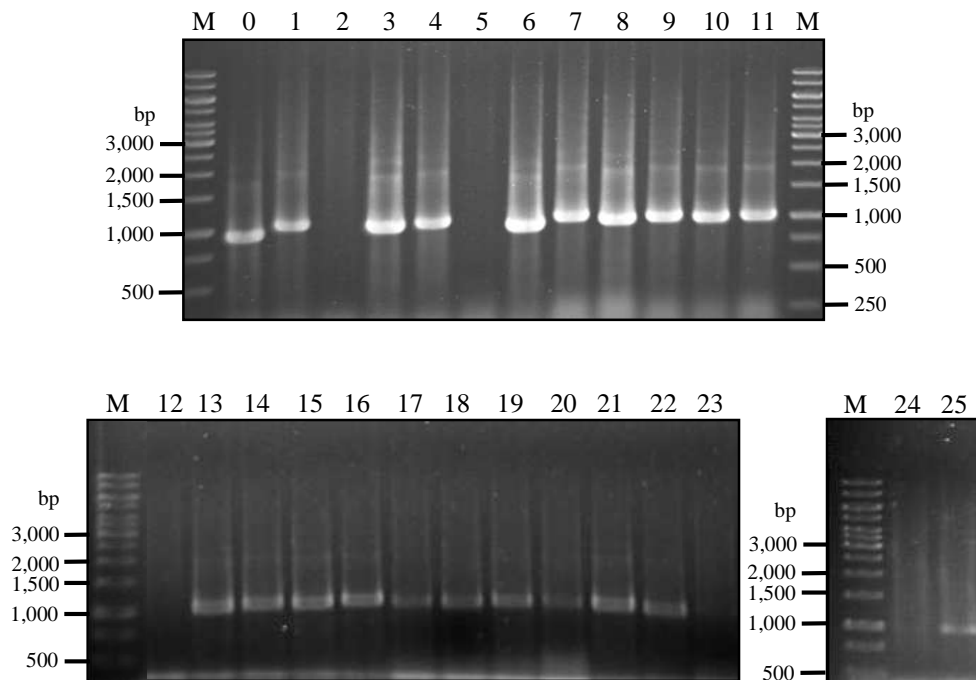
**Figure 68** Results of the HuScFv expression and Western blot analysis of the 19 *huscFv*-pCANTAB5E-transformed HB2151 *E. coli* colonies derived from single round of bio-panning with **P5** which principally contained phospholipase A2.

Lanes M, standard protein markers

Whole cell lysates of 19 HB2151 *E. coli* colonies carrying *huscFv* (lanes 1-7 and 9-20 of **Figure 67**) could express HuScFv (100%) at relative molecular masses (*Mr*) ~25-27 (lanes 1-7 and 9-20)

Numbers at the left of both block of the figure are relative molecular mass (*Mr*)

\*Some HuScFv appeared as multiple protein bands because the HuScFv may be mature (without signal peptide) or immature (with signal peptide) and some proteins were degraded

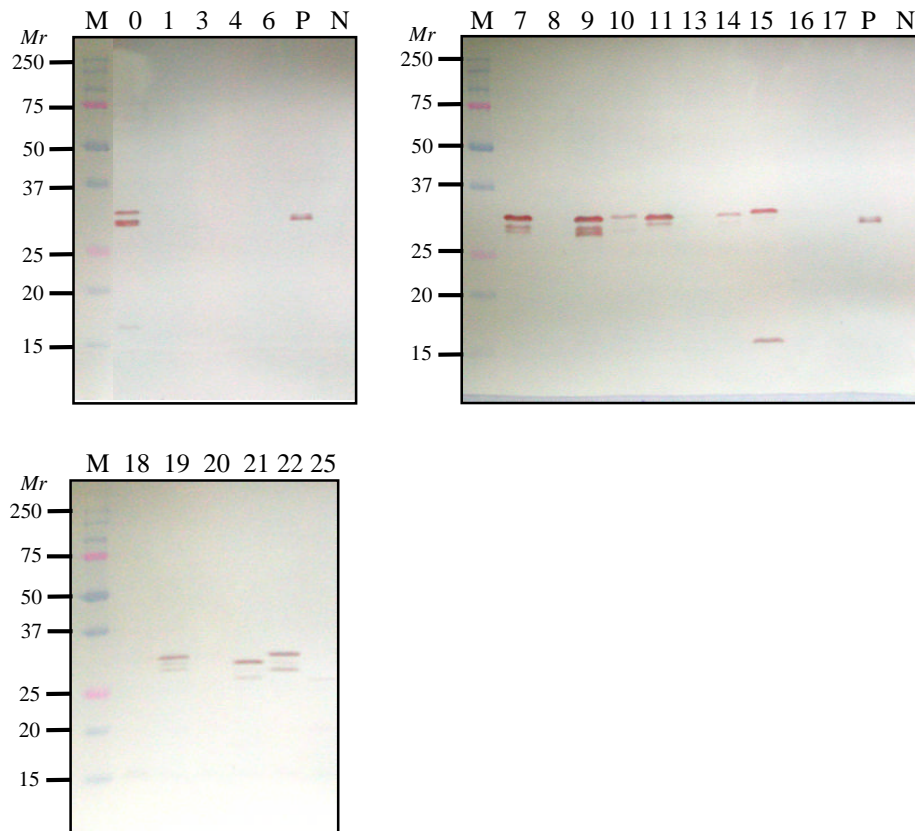


**Figure 69** Results of the *huscFv* amplification of the 26 transformed HB2151 *E. coli* colonies derived from the first single round of bio-panning with **P8** (principally contained long neurotoxin).

Lanes M, GeneRuler 1 kb DNA ladder

Lanes 0-25, the *huscFv* amplicons of the HB2151 *E. coli* clones no. 1-25, respectively

Numbers at the left of three blocks of the figure are DNA sizes in bp



**Figure 70** Results of the HuScFv expression and Western blot analysis of the 21 *huscFv*-pCANTAB5E-transformed HB2151 *E. coli* colonies derived from single round of bio-panning with **P8**.

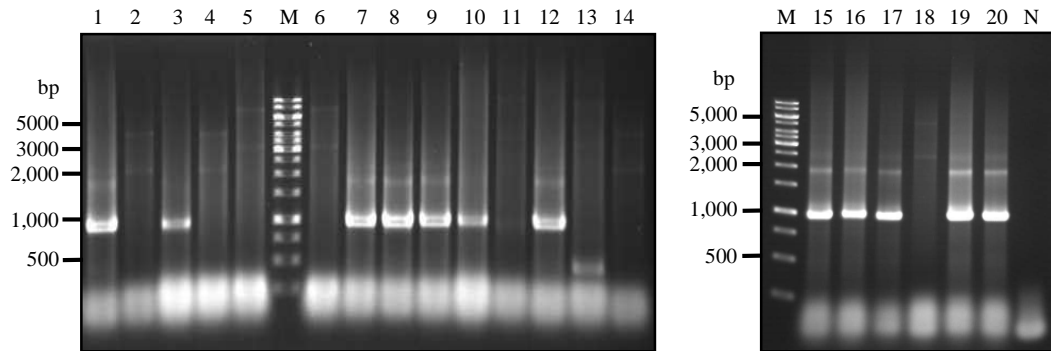
Lanes M, standard protein markers

Whole cell lysates of 10 transformed HB2151 *E. coli* colonies (48%) from the 21 colonies of *huscFv*-carrying HB2151 *E. coli* (lanes 0, 1, 3, 4, 6-11, 13-22 and 25 of **Figure 69**) could express HuScFv at  $Mr \sim 25-27$  (lanes 0, 7, 9-11, 14, 15, 19, 21 and 22)

Lane P, whole cell lysate of HB2151 *E. coli* harboring mouse *scFv*-phagemid vector (positive control).

Lane N, whole cell lysate of HB2151 *E. coli* harboring pUC19 (negative control)

Numbers at the left of the three blocks are relative molecular masses ( $Mr$ )



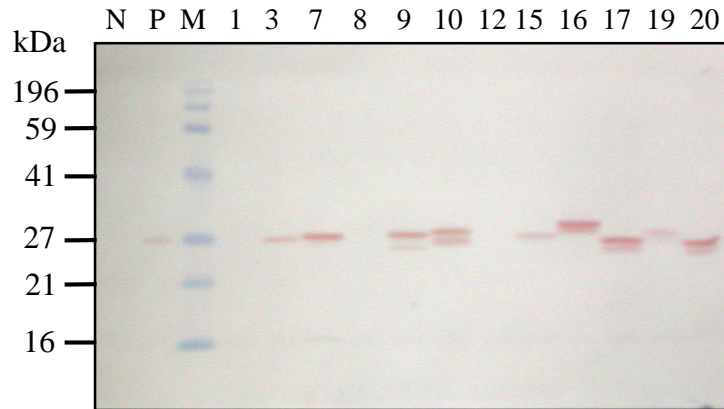
**Figure 71** Results of the *huscFv* amplification of the 20 transformed HB2151 *E. coli* colonies derived from the second single round of bio-panning with **P8**.

Lanes M, GeneRuler 1 kb DNA ladder

Lanes 1-20, the *huscFv* amplicons of the HB2151 *E. coli* clones no. 1-20, respectively

Lane N is negative control, no DNA template in PCR mixture

Numbers at the left of both blocks are DNA sizes in bp



**Figure 72** Results of the HuScFv expression and Western blot analysis of the 12 *huscFv*-pCANTAB5E-transformed HB2151 *E. coli* colonies derived from single round of bio-panning (2<sup>nd</sup> time) with **P8**.

Lanes M, standard protein markers

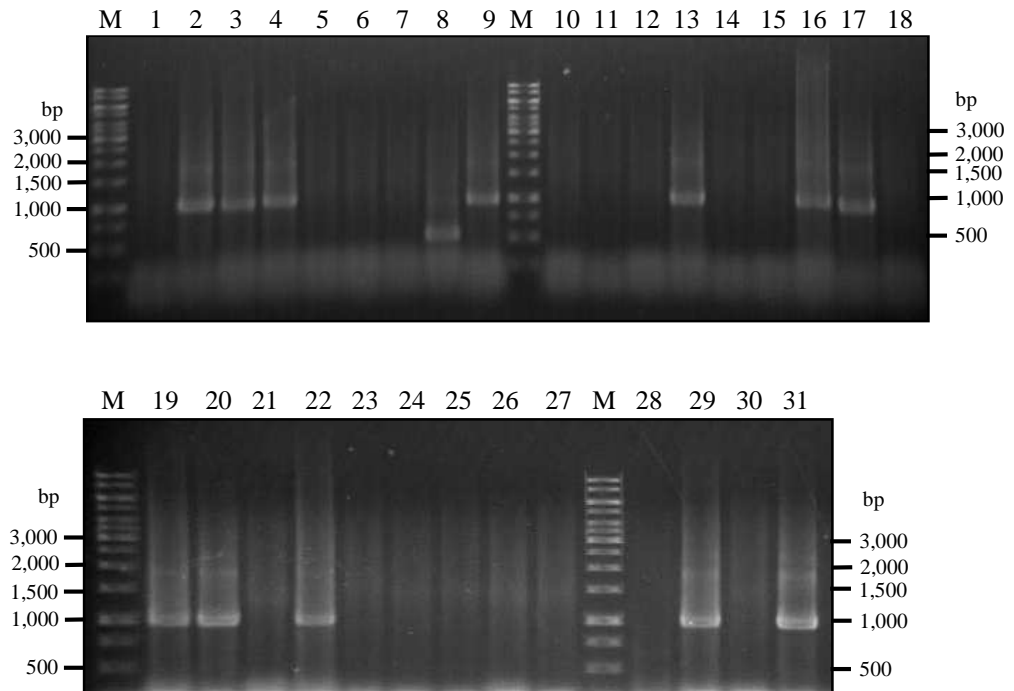
Whole cell lysates of 9 transformed HB2151 *E. coli* colonies (75%) from the 12 *huscFv*-carrying HB2151 *E. coli* colonies (lanes no. 1, 3, 7-10, 12, 15-17, 19, and 20 of **Figure 71**) could express HuScFv seen at relative molecular masses (*Mr*) ~25-27 (lanes 3, 7, 9, 10, 15-17, 19 and 20)

Lane P, whole cell lysate of HB2151 *E. coli* harboring mouse *scFv*/phagemid vector (positive control)

Lane N, whole cell lysate of HB2151 *E. coli* harboring pUC19 (negative control)

Numbers at the left of the figure are relative molecular masses (*Mr*).

\*Some HuScFv appeared as multiple protein bands because the HuScFv may be mature (without signal peptide) or immature (with signal peptide) and some proteins were degraded

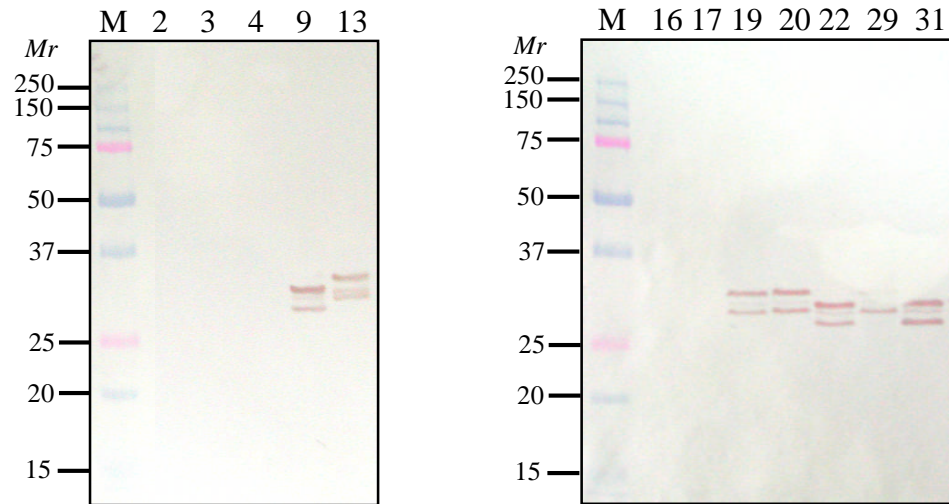


**Figure 73** Results of the *huscFv* amplification of the 31 transformed HB2151 *E. coli* colonies derived from the third round of bio-panning with **P8**.

Lanes M, GeneRuler 1 kb DNA ladder

Lanes 1-31, the *huscFv* amplicons of HB2151 *E. coli* clones no. 1-31, respectively

Numbers at the left and right of both blocks are DNA sizes in bp



**Figure 74** Results of the HuScFv expression and Western blot analysis of the 12 *huscFv*-pCANTAB5E-transformed HB2151 *E. coli* colonies derived from third round of bio-panning with **P8**.

Lanes M, standard protein markers

Whole cell lysates of 7 HB2151 *E. coli* colonies (58%) from 12 *huscFv*-carrying HB2151 *E. coli* colonies (lanes no. 2-4, 9, 13, 16-17, 19-20, 22, 29, and 31 of **Figure 73**) could express HuScFv seen at relative molecular masses (*Mr*) ~25-27 (lanes 9, 13, 19, 20, 22, 29, and 31)

Numbers at the left of both blocks are relative molecular masses (*Mr*)

\*Some HuScFv appeared as multiple protein bands because the HuScFv may be mature (without signal peptide) or immature (with signal peptide) and some proteins were degraded

## **10. HuScFv-ELISA for detecting the binding of the HuScFv to the venom proteins**

The crude *N. kaouthia* venom was used to screen HB2151 *E. coli* clones that produced venom protein-specific HuScFv.

### **10.1 HuScFv derived from the single round of bio-panning with P1 (cobra venom factor)**

Whole cell lysates of the eight HB2151 *E. coli* clones expressing HuScFv (**Figure 60**, lanes 1-5 and 7-9) were used in the indirect ELISA. Five HB2151 *E. coli* clones, *i.e.*, P1/1, P1/2, P1/3, P1/5, and P1/9, showed ELISA signal at  $A_{405\text{nm}}$  with crude Thai cobra venom more than 0.05 and two times higher than the ELISA signal of the skim milk which was used as negative antigen control (**Figure 75**).

### **10.2 HuScFv derived from the single round of bio-panning with P3 (phospholipase A2)**

Whole cell lysates of the HB2151 *E. coli* clones, both expressed and did not express HuScFv (**Figure 62**, lanes 2, 3, 5-7, 10, 11, 14, 18-20, 22, 23, 26, 27, 30, 36 and 37) were used in the indirect ELISA (**Figure 76**, lanes P3/2, P3/3, P3/5-P3/7, P3/10, P3/11, P3/14, P3/18-P3/20, P3/22, P3/23, P3/26, P3/27, P3/30, P3/36, and P3/37, respectively). Five HB2151 *E. coli* clones, *i.e.*, P3/2, P3/5, P3/6, P3/7, and P3/18, showed ELISA signal at  $A_{405\text{nm}}$  with crude Thai cobra venom more than 0.05 and two times higher than the ELISA signal of the skim milk (**Figure 76**).

### **10.3 HuScFv derived from the single round of bio-panning with P4 (natrin)**

Whole cell lysates of the nine HB2151 *E. coli* clones, that expressed HuScFv (**Figure 64**, lanes 1, 3, 5, 8, 10, 12, 16, 18, and 21), were used in the indirect ELISA (**Figure 77**, lanes P4/1, P4/3, P4/5, P4/8, P4/10, P4/12, P4/16, P4/18, and P4/21 respectively). Two HB2151 *E. coli* clones, *i.e.*, P4/8 and P4/21, showed ELISA signal at  $A_{405\text{nm}}$  with crude Thai cobra more than 0.05 and two times higher than ELISA signal with skim milk (**Figure 77**).

#### 10.4 HuScFv derived from the third rounds of bio-panning with P4 (natrin)

Whole cell lysates of four HB2151 *E. coli* clones, that expressed HuScFv (**Figure 66**, lanes 3, 12, 13 and 17), were used in the indirect ELISA (**Figure 78**, P4/3, P4/12, P4/13, and P4/17, respectively). None of the HB2151 *E. coli* clones showed significant ELISA signal at  $A_{405\text{nm}}$  with crude Thai cobra venom (more than 0.05 and two times higher than ELISA signal with skim milk) (**Figure 78**).

#### 10.5 HuScFv derived from single round of bio-panning with P5 (phospholipase A2)

Whole cell lysates of all of the 20 HB2151 *E. coli* colonies, both expressed and did not express HuScFv (**Figure 68**, lanes 1-20), were used in the indirect ELISA (**Figure 79**, P5/1-P5/20, respectively). Ten HB2151 *E. coli* clones, *i.e.*, P5/1, P5/2, P5/3, P5/4, P5/5, P5/6, P5/7, P5/9, P5/10 and P5/11, showed ELISA signal at  $A_{405\text{nm}}$  with crude Thai cobra venom more than 0.05 and two times higher than ELISA signal with skim milk (**Figure 79**).

#### 10.6 HuScFv derived from bio-panning with P8 (neurotoxin)

##### A. The first single round panning

Whole cell lysates of 10 HB2151 *E. coli* clones, that expressed HuScFv (**Figure 70**, lanes 0, 7, 9, 10, 11, 14, 15, 19, 21 and 22), were used in the indirect ELISA (**Figure 80**, lanes P8/0, P8/7, P8/9, P8/10, P8/11, P8/14, P8/15, P8/19, P8/21 and P8/22, respectively). Three HB2151 *E. coli* clones, *i.e.*, P8/0, P8/9, and P8/19, showed ELISA signal at  $A_{405\text{nm}}$  with crude Thai cobra venom more than 0.05 and two times higher than ELISA signal with skim milk (**Figure 80**).

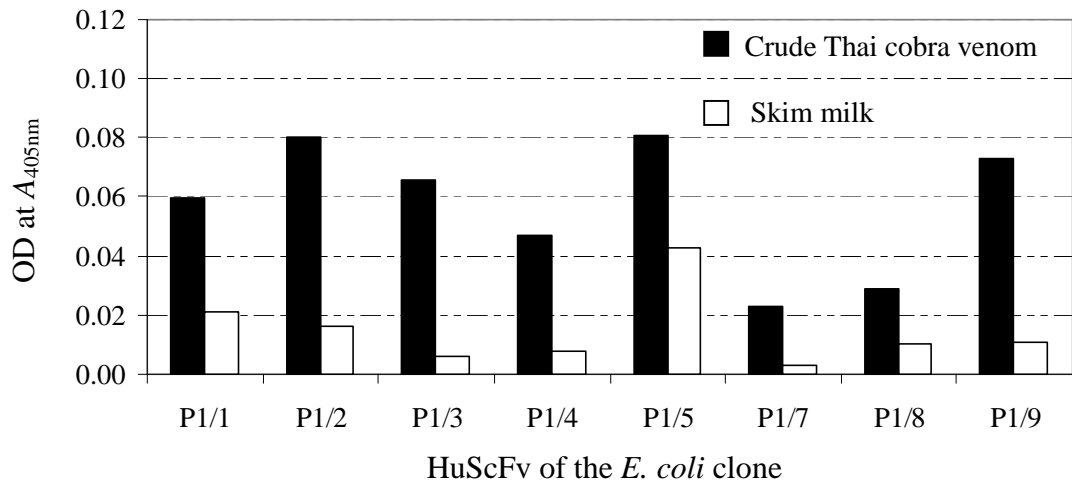
##### B. The second single round panning

Whole cell lysates of 9 HB2151 *E. coli* colonies, that expressed HuScFv (**Figure 72**, lanes 3, 7, 9, 10, 15, 16, 17, 19 and 20), were used in the indirect ELISA (**Figure 81**, lanes P8/3, P8/7, P8/9, P8/10, P8/15, P8/16, P8/17, P8/19, and P8/20 respectively). Two HB2151 *E. coli* clones, *i.e.*, P8/7 and P8/10, showed significant ELISA signal at  $A_{405\text{nm}}$  (more than 0.05 and two times higher than ELISA signal with skim milk) (**Figure 81**).

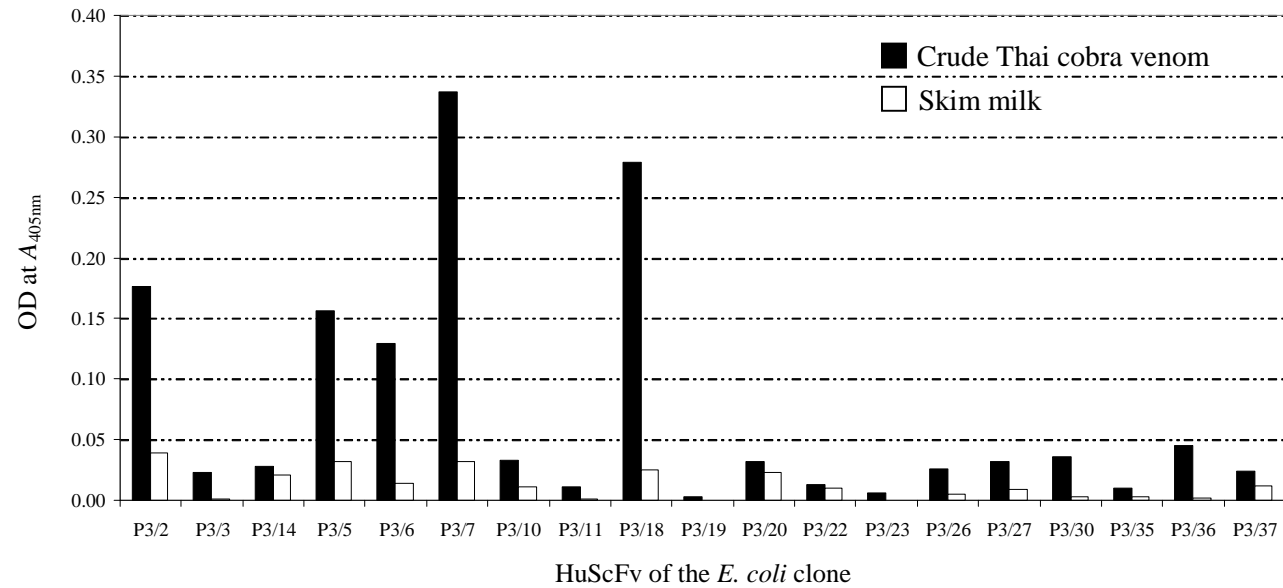
### C. The third rounds

Whole cell lysates of 9 HB2151 *E. coli* clones, that expressed HuScFv (**Figure 74**, lanes 9, 13, 19, 20, 22, 29 and 31), were used in the indirect ELISA (**Figure 82**, lanes P8/9, P8/13, P8/19, P8/20, P8/22, P8/29 and P8/31, respectively). Two HB2151 *E. coli* clones, *i.e.*, P8/22 and P8/31, showed significant ELISA signal at  $A_{405\text{nm}}$  with crude Thai cobra venom (more than 0.05 and two times higher than ELISA signal with skim milk) (**Figure 82**).

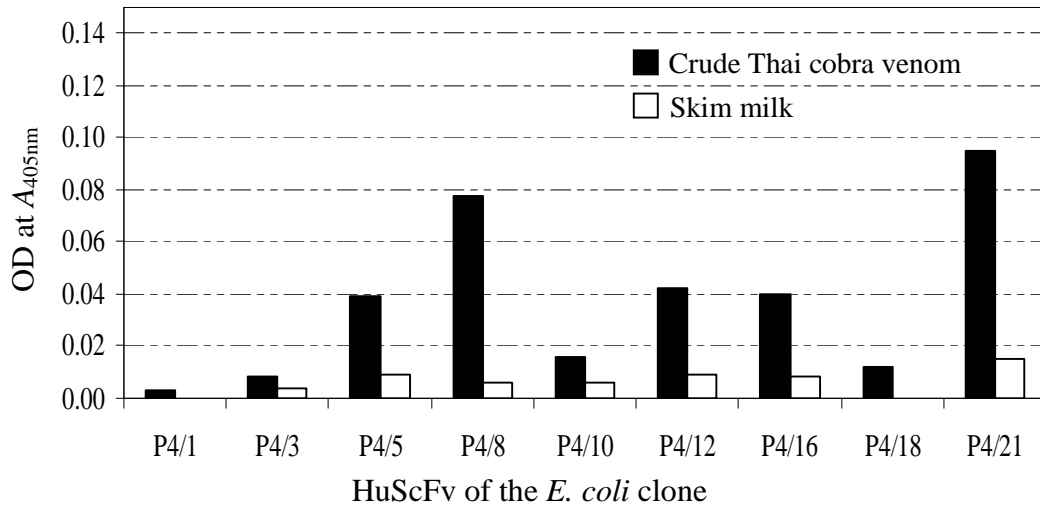
The HuScFv-expressing HB2151 *E. coli* colonies that showed  $A_{405\text{nm}}$  more than 0.05 and two times higher than ELISA signal with skim milk were selected for HuScFv production and purification which were used for the *in vivo* toxin neutralization test.



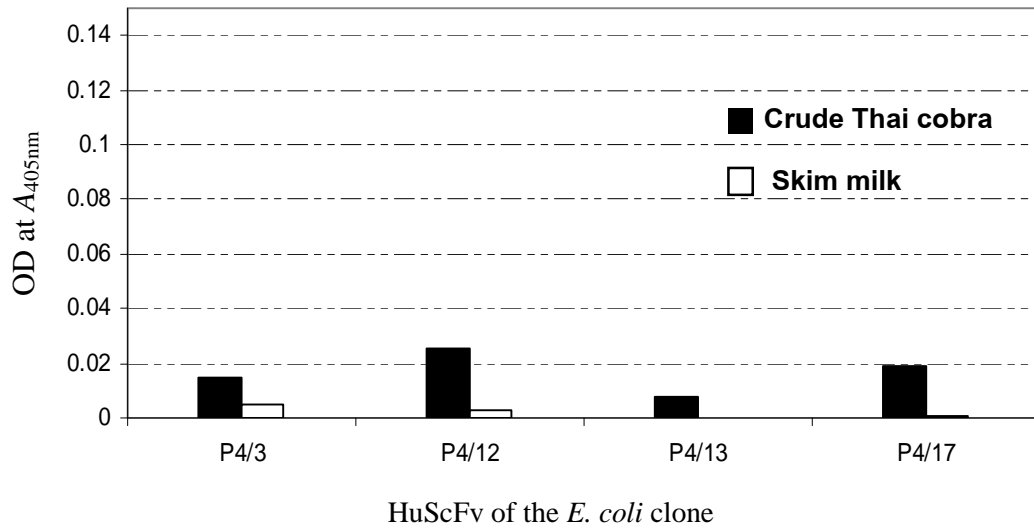
**Figure 75** Results of the indirect ELISA of the HuScFv of the *E. coli* clones derived from single round of bio-panning with **P1** (cobra venom factor).



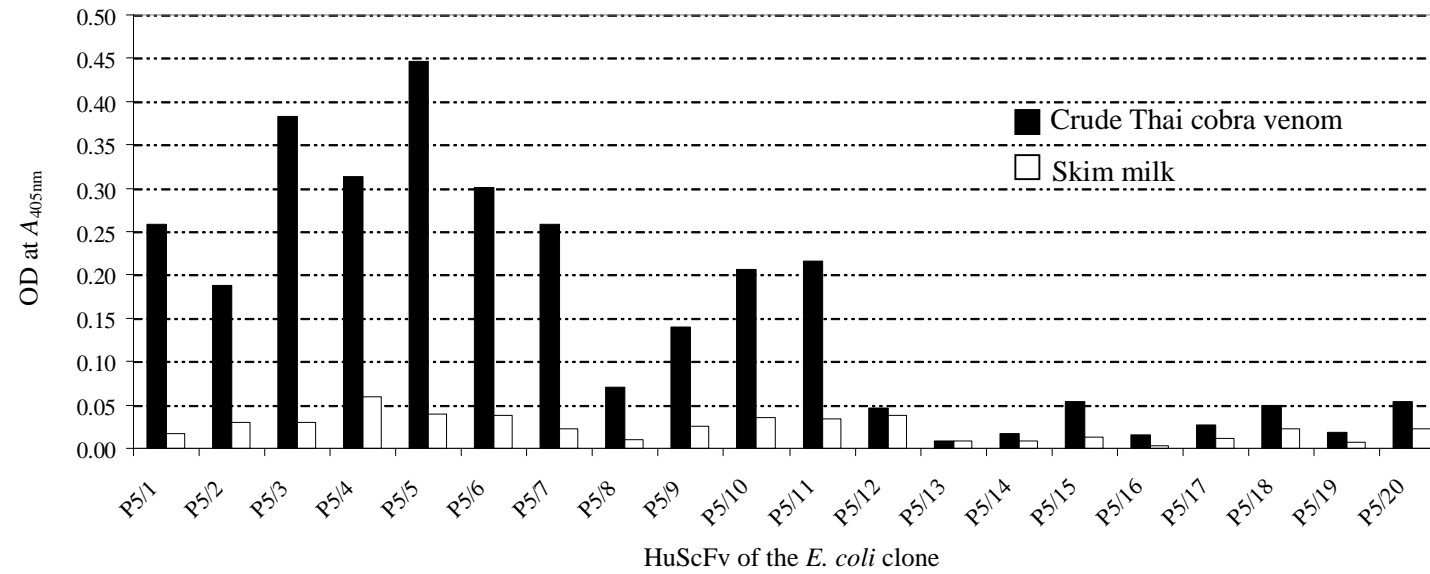
**Figure 76** Results of the indirect ELISA of HuScFv of *E. coli* clones derived from the single round bio-panning with **P3** (phospholipase A2).



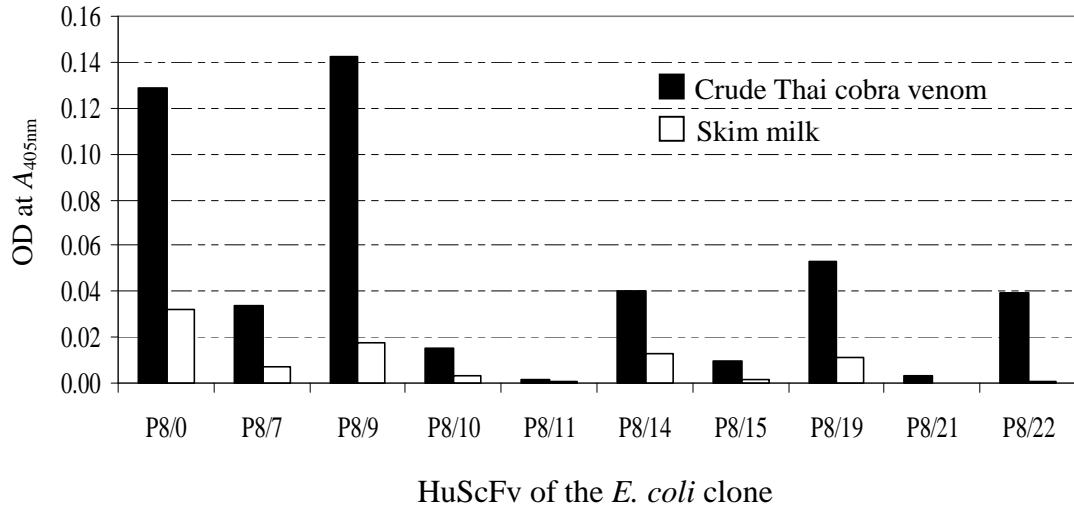
**Figure 77** Result of the indirect ELISA of HuScFv of *E. coli* clones derived from single round of bio-panning with **P4** (natrin).



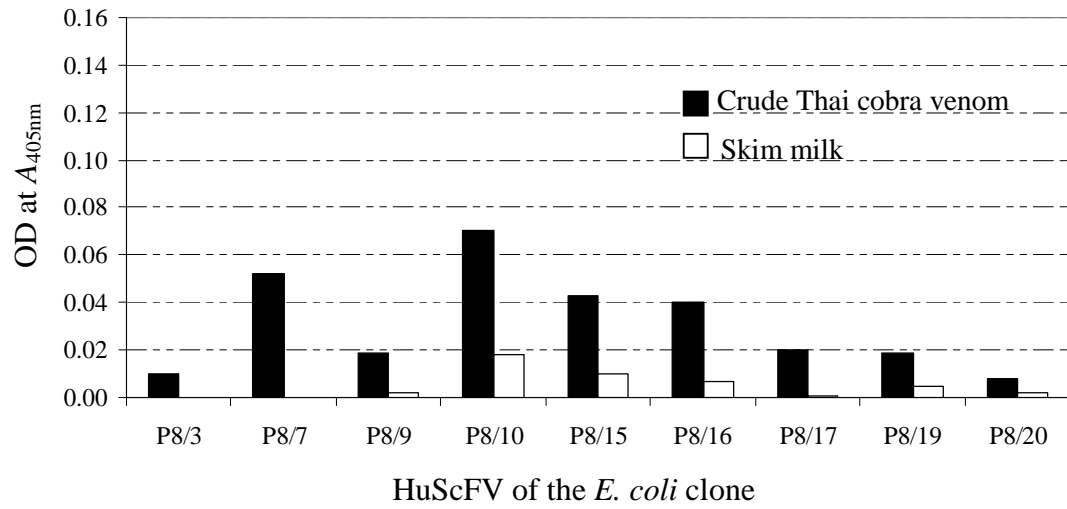
**Figure 78** Results of the indirect ELISA of HuScFv of the *E. coli* clones derived from the third rounds bio-panning with **P4** (natrin).



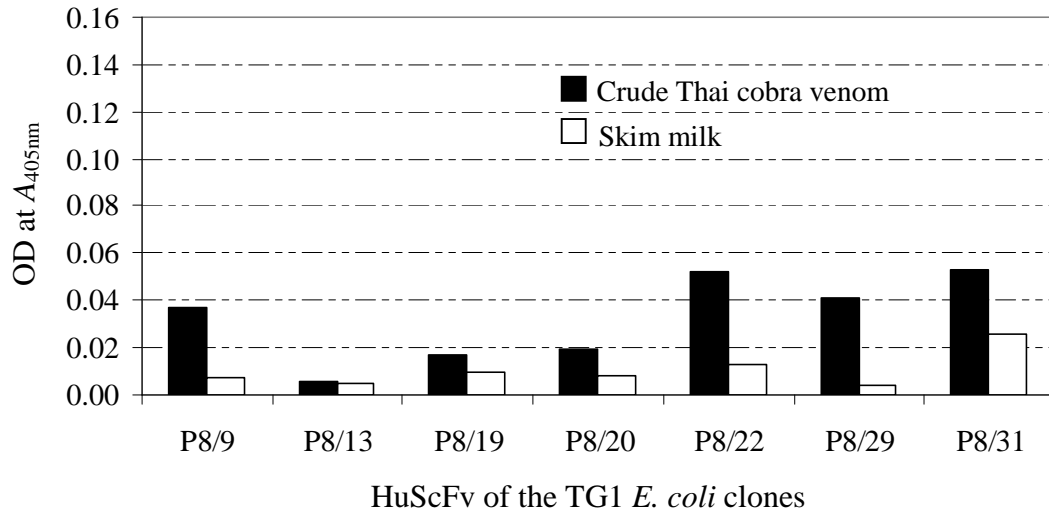
**Figure 79** Results of the indirect ELISA of HuScFv derived from the single round bio-panning with P5 (phospholipase A2).



**Figure 80** Results of the indirect ELISA of HuScFv derived from the first single round of bio-panning with **P8** (neurotoxin).



**Figure 81** Indirect ELISA of HuScFv derived from the second time of single round of bio-panning with **P8** (neurotoxin).



**Figure 82** Indirect ELISA of HuScFv derived from the third rounds bio-panning with **P8** (neurotoxin)

## **11. Results of dot-ELISA and Western blot analysis for detecting the HuScFv binding to the venom components**

The soluble human ScFv prepared from HB2151 *E. coli* clones that gave positive results in indirect ELISA with crude Thai cobra venom, were tested for their binding with column-separated protein components (P1-P11) of Thai cobra venom by dot-ELISA and gel-separated crude venom by WB.

### **11.1 Dot-ELISA**

#### **A. HuScFv of *E. coli* clones which were derived from bio-panning with P1 (cobra venom factor) are shown in Figure 83 and summarized as follows:**

1. HuScFv of clone P1/1 reacted with protein components in P1, P6, and P8.
2. HuScFv of clone P1/2 reacted with protein components in P1, P6, P7, P8, P9 and P10.
3. HuScFv of clone P1/3 weakly reacted with protein components in P1, P6 and P8.
4. HuScFv of clone P1/1 weakly reacted with protein components in P1, P6, and P8.
5. HuScFv of clone P1/9 reacted with protein components in P1, P6, P8, P9, and P10.

#### **B. HuScFv of *E. coli* clones which were derived from bio-panning with P3 (phospholipase A2) are shown in Figure 84 and summarized as follows:**

1. HuScFv of clone P3/2 reacted with protein components in P1 and reacted weakly with P5, P6, P7, P8, P9, and P10.
2. HuScFv of clone P3/5 could not react with any protein components in P1 to P11.
3. HuScFv of clone P3/6 reacted with protein components in P1 and weakly reacted with P5, P6, P7, P8, P9 and P10.
4. HuScFv of clone P3/7 reacted with protein components in P1 and weakly reacted with P5, P6, P7, P8, P9, and P10.
5. HuScFv of clone P3/18 could not react with any protein component in P1 to P11.

**C. HuScFv of *E. coli* clones derived from bio-panning with P4 (natrin)**

HuScFv of clones P4/8 and 4/21 could not react with any protein component in P1 to P11 as shown in **Figure 84**.

**D. HuScFv of *E. coli* clones derived from bio-panning with P5**

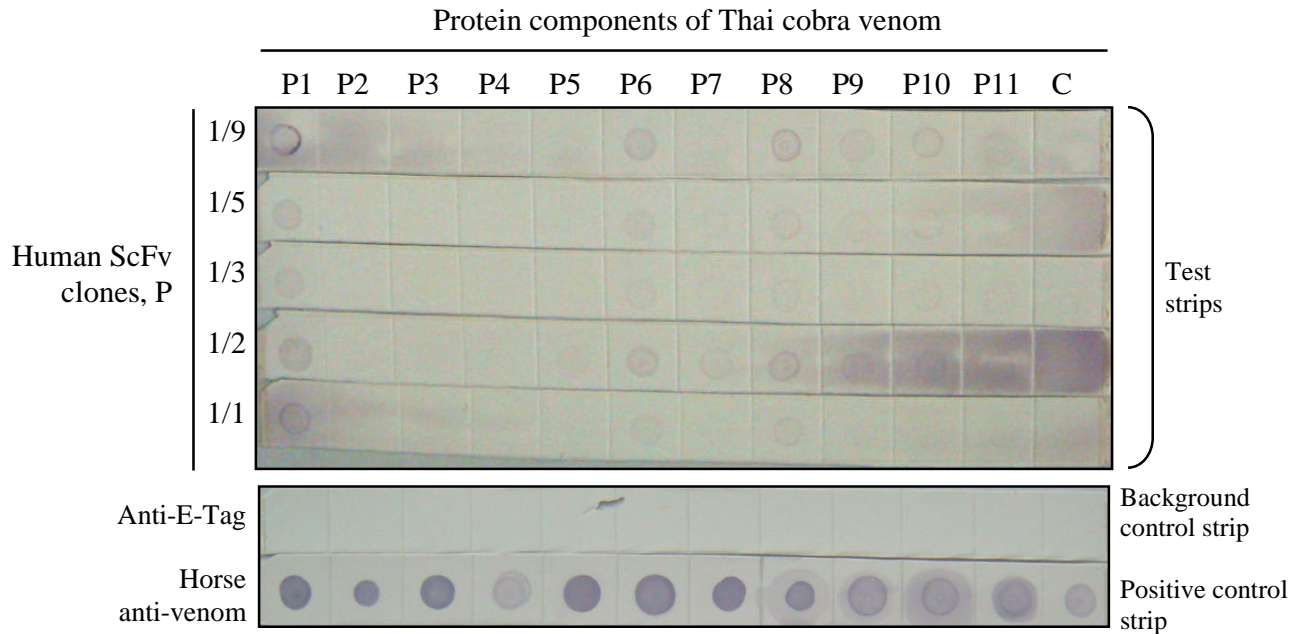
(**phospholipase A2**) are shown in **Figure 86** and summarized as follows:

1. HuScFv of clones P5/1, P5/2, P5/3, P5/4, and P5/5 reacted with protein components in P1, P6, and P8.
2. HuScFv of clone P5/6 reacted with protein components in P1, P6, P8, P9, and P10.
3. HuScFv of clone P5/7 reacted with protein components in P1, P6, P7, P8, P9, and P10.
4. HuScFv of clone P5/9 reacted with protein components in P1, P6, P8, P9, and P10.
5. HuScFv of clone P5/10 reacted with protein components in P6, P8, and P9.
6. HuScFv of clone P5/11 reacted with protein components in P6, P8, and P9.

**E. HuScFv of *E. coli* clones derived from bio-panning with P8 (neurotoxin)**

are shown in **Figure 87** and summarized as follows:

1. HuScFv of clone P8/0/1 reacted with protein components in P1, P8, P10, and P11.
2. HuScFv of clone P8/9/1 reacted with protein components in P8 and P10.
3. HuScFv of clone P8/19/1 reacted with protein components in P8, P10, and P11.
4. HuScFv of clone P8/7/2 reacted with protein components in P8, P9, P10, and P11.
5. HuScFv of clone P8/10/2 reacted with protein components in P1, P8, P9, P10, and P11.
6. HuScFv of clone P8/22/3 reacted weakly with only protein component in P8.
7. HuScFv of clone P8/31/3 reacted with protein components in fraction P1, P8, P10, and P11.

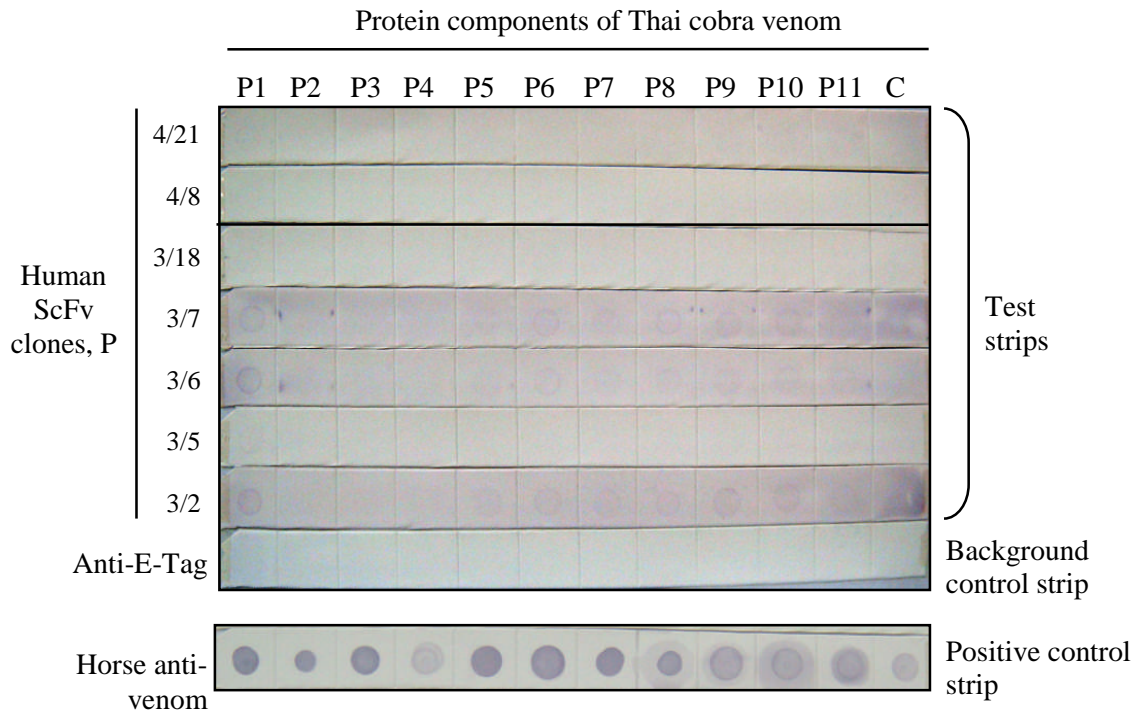


**Figure 83** Dot-ELISA results of soluble HuScFv prepared from *E. coli* HB2151 colonies derived from bio-panning with **cobra venom factor** in P1 of Thai cobra venom, namely clones P1/1, P1/2, P1/3, P1/5, and P1/9.

Eleven protein fractions (P1-P11) purified by ion exchange chromatography and crude Thai cobra venom (C) were dotted onto seven NC strips and the five strips were individually incubated with HuScFv from clones P1/9, P1/5, P1/3, P1/2, and P1/1 (Test strips), respectively.

Background control NC piece was reacted with anti-E-Tag MAb

Positive control strip was reacted with horse anti-Thai cobra venom

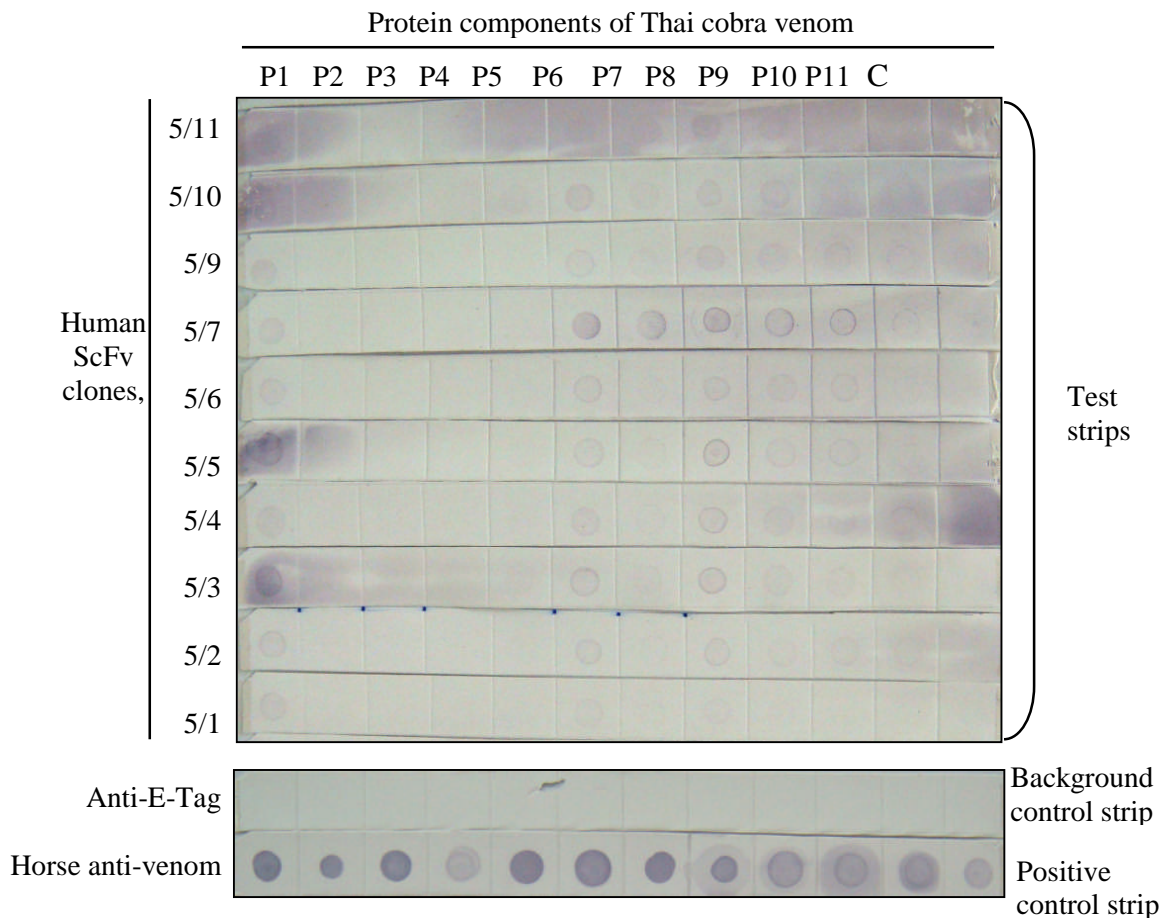


**Figure 84** Dot-ELISA results of soluble HuScFv prepared from *E. coli* HB2151 colonies derived from bio-panning with **phospholipase** in P3 of Thai cobra venom namely P3/2, P3/5, P3/6, P3/7, and P3/18 and **natrin** in P4 of Thai cobra venom, namely P4/8 and P4/21.

Eleven protein fractions (P1-P11) purified by ion exchange chromatography and crude Thai cobra venom (C) were dotted onto nine strips of NC. Seven strips were individually incubated with HuScFv from clones P4/8, P4/21, P3/8, P3/7, P3/6, P3/5, and P3/2 (Test strips), respectively.

Background control NC was reacted with anti-E-Tag MAb

Positive control strip was reacted with horse anti-Thai cobra venom

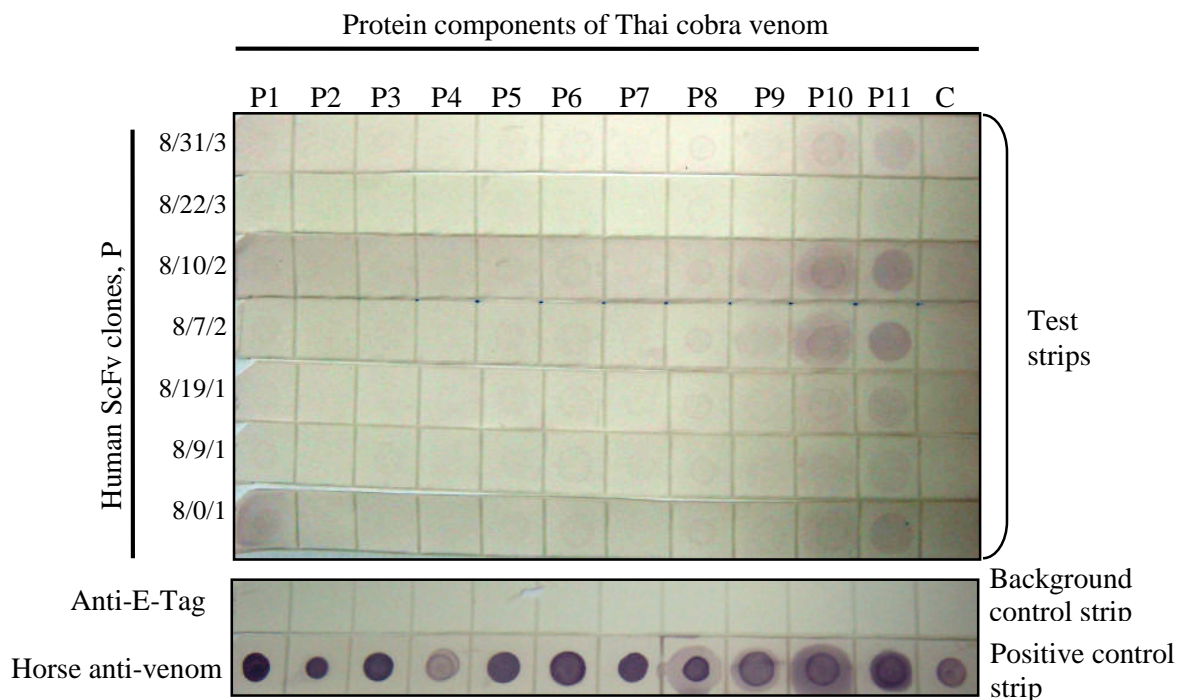


**Figure 85** Dot-ELISA results of soluble HuScFv prepared from *E. coli* HB2151 derived from bio-panning with **phospholipase A2** in P5 of Thai cobra venom, namely P5/1, P5/2, P5/3, P5/4, P5/5, P5/6, P5/7, P5/9, P5/10, and P5/11.

Eleven protein fractions (P1-P11) purified by ion exchange chromatography and crude Thai cobra venom (C) were dotted onto 12 NC strips. Ten strips were individually incubated with HuScFv from clones P5/11, P5/10, P5/9, P5/7, P5/6, P5/5, P5/4, P5/3, P5/2, and P5/1 (Test strips), respectively.

Background control NC was reacted with anti-E-Tag MAb

Positive control strip was reacted with horse anti-Thai cobra venom



**Figure 86** Dot-ELISA results of soluble HuScFv prepared from *E. coli* HB2151 derived from bio-panning with neurotoxin in P8 of Thai cobra venom, namely P8/0/1, P8/9/1, P8/19/1, P8/7/2, P8/10/2, P8/22/3, and P8/31/3.

Eleven protein fractions (P1-P11) purified by ion exchange chromatography and crude Thai cobra venom (C) were dotted onto nine strips of NC membranes. Seven strips were individually incubated with HuScFv from clones P8/31/3, P8/22/3, P8/10/2, P8/7/2, P8/19/1, P8/9/1, and P8/0/1 (Test strips), respectively.

Background control NC was reacted with anti-E-Tag MAb

Positive control strip was reacted with horse anti-Thai cobra venom